Université de Liège

Faculté des Sciences

DOP
DEPARTAMENTO DE OCEANOGRAFIA E PESCAS
UNIVERSIDADE DOS AÇORES

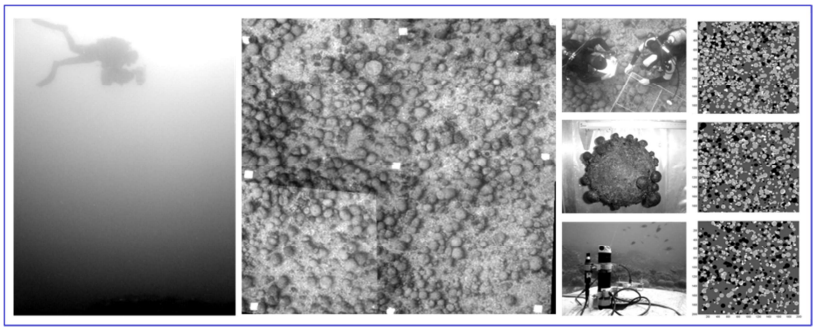
Study of the ecology, population structure and dynamic of the macroalgae *Codium elisabethae* in Faial (Azores) with underwater visible imagery.

Damien SIRJACOBS

Laboratory of Oceanology
Department of Sciences and Management of the Environment - ULG

Department of Oceanography and Fisheries
University of the Azores

Laboratory of Algology, Mycology and Experimental Systematics
Department of Life Sciences – ULG



Thesis submitted for the degree of
Docteur en Sciences

August 2011

Abstract

Codium elisabethae O.C. Schmidt is a dark green globose macroalgae isolating an internal sea water volume in a lumen. *Codium elisabethae* is endemic to the Macaronesian region and is very similar to *Codium bursa* C. Agardh whose distribution range spans the West-European, North-Western African and Mediterranean coasts and which was proposed as a potential indicator of coastal environmental changes based on the study of its ecology, revealing its long lifespan. Until recently, relatively little was known on *Codium elisabethae* as compared to the more widespread *Codium bursa*.

To fill this gap, the present research aimed at producing an accurate description of the ecology and population dynamics of *Codium elisabethae* occupying the rocky shores of the Monte da Guia Special Area of Conservation (SAC)/Natura 2000 network (Faial, Azores). To achieve this, two reference sites were selected for long term monitoring: a sheltered no-go reserve exhibiting a dense *Codium elisabethae* population (Caldeirinhas), and a location experiencing more exposed conditions holding a sparser population (Ponta Furada).

First, environmental conditions experimented by benthic organisms were extensively quantified and interpretated in regard to topographical particularities of each site. The study of reproduction dynamics showed a persistent summer fertility and an important vegetative reproduction. Important nutrient concentration ratio was found between the *Codium elisabethae* lumen water and surrounding sea water (mean ratios: nitrates: 5.7; ammonium: 3.4; phosphates: 3.1). *In situ* counting's and size measurements revealed much higher densities of young recruits in the site of the Caldeirinhas (order of 20 ind/m²) than in the one of Ponta Furada (order of 1/m), for both summer and winter.

Secondly, underwater visible imagery was exploited as an efficient and non-invasive alternative to classical *in situ* population estimation. Between August 2003 and November 2005, fifteen seabed photo coverages were collected by scuba-divers. Subsequent image processing consisted in mosaicing, interactive identification, and automatic change detection methods. This allowed quantifying the seasonal fluctuations of population structures (density, percentage cover and biomass) and of population dynamics (growth, recruitment, mortality and primary production). Chi-square tests of image-derived estimates and *in situ* measurements confirmed the validity of a centimeter precision for the estimation of population structure of individuals above 4 cm diameter. Important variability of population structure and density was observed within the sites at small spatial scales. Significant differences of population structure and dynamics parameters are demonstrated between two close-by but contrasting coastal habitats. Population density showed a sharp reduction in autumn 2003 and did not recover fully in spring and summer 2004. During the following year, population of the protected site maintained density and biomass, while at the exposed site population density dropped. In contrast with conclusions from earlier studies on the Azorian *Codium elisabethae* and on the Mediterranean *Codium bursa*, the present study revealed higher biomass (34 - 730 g dry wt.m^{-2}), growth rates (up to 2.5 cm/month in summer) and primary production (0.53 – 11.5 g dry wt.m^{-2}.day^{-1}), and demonstrated the seasonal fluctuations of these parameters for the studied Azorian *Codium elisabethae* population. The lifespan of *Codium elisabethae* was estimated to reach at least 7 years in the SAC of Monte da Guia based on an integration of average seasonal growth rates measured by imagery on extended population samples.

This study demonstrates the high potential of registered underwater photomosaics time-series for long term surveys of macroalgae populations. This work provides also a strong framework to further developments and applicability to other species, which should be helpful to strengthen our current understanding of benthic ecosystem processes.

Résumé

Codium elisabethae O.C. Schmidt est une macroalgue sphérique dont le thalle vert foncé englobe et isole un volume d'eau de mer dans un lumen. *Codium elisabethae* est endémique de la Macaronésie et est très semblable à *Codium bursa* C. Agardh dont l'aire de répartition couvre les côtes Ouest-Européennes, Nord-Ouest Africaines et Méditerranéennes et qui fut proposée comme indicateur potentiel de changements environnementaux côtiers sur base de l'étude de son écologie et de sa grande longévité. Jusqu'à récemment, *Codium elisabethae* était mal connue en comparaison à l'algue plus commune *Codium bursa*.

Cette recherche vise une description précise de l ' écologie et dynamique des populations de *Codium elisabethae* au sein des habitats rocheux du site de Monte da Guia (SAC/ Réseau Natura 2000) (Faial, Azores). Pour cela, deux sites de référence ont été sélectionnés pour une étude à long terme : une réserve en protection intégrale présentant une population dense (Caldeirinhas), et une zone plus exposée présentant une population plus éparse (Ponta Furada).

D'une part, les conditions environnementales subies par les organismes benthiques y sont quantifiées de façon détaillée et interprétées en fonction des particularités topographiques des sites. Une étude de dynamique de reproduction a démontré une fertilité persistante durant l ' été et une reproduction végétative importante. Un important rapport de concentration en nutriments est mesuré entre l'eau contenue dans le lumen et l ' eau environnante (rapports moyens: nitrates: 5.7; ammonium: 3.4; phosphates: 3.1). Des comptages *in situ* et des mesures de dimension ont clairement indiqué une densité plus élevé de jeunes recrues dans le site des Caldeirinhas (ordre de 20 indiv.m^{-2}) que dans celui de Ponta Furada (ordre de 1 indiv. m^{-2}), été comme hiver.

D'autre part, l'imagerie visible sous-marine est exploitée en tant qu'alternative efficace et non intrusive à l'estimation classique de ces populations. Entre août 2003 et novembre 2005, quinze couvertures photographiques enregistrées en plongée dans les sites furent exploitées pour l'analyse de dynamique de population. La constitution de mosaïques d'images suivies d'identification interactive puis de détection automatique de changements ont permis de quantifier les fluctuations saisonnières des structures de population (densité, pourcentage de couverture, biomasse) et des dynamiques de population (croissance, recrutement, mortalité et production primaire). Des tests de contingence (Chi-carré) comparant estimations obtenues à partir d'images et mesures *in situ* confirment la validité d'une quantification au centimètre de la structure de population pour les individus d'un diamètre supérieur à 4 cm. Une importante variabilité de la structure de population et de la densité fut observée à l'intérieur des sites à petite échelle spatiale. Des différences significatives de structure et dynamique de population sont démontrées entre deux habitats côtiers proches mais différents. La densité de population chute fortement durant l'automne 2003 et n'a pas montré une complète récupération au cours du printemps et de l'été 2004. L'année suivante, densité et biomasse de la population du site protégé se sont maintenues, à l'inverse de la densité dans le site exposé. En comparaison aux conclusions des études précédentes concernant *Codium elisabethae* aux Azores et *Codium bursa* en Méditerranée, l'étude actuelle révèle une biomasse (34 – 730 g m.s. m^{-2}), un taux de croissance (jusque 2.5cm par mois en été) et une production primaire (0.53 – 11.5 g m.s. $m^{-2}.jour^{-1}$) plus élevés et a démontré des fluctuations saisonnières de ces paramètres pour *Codium elisabethae* aux Açores. L'intégration des taux de croissance saisonniers mesurés par imagerie sur de larges échantillons de population a démontré que la durée de vie de *Codium elisabethae* pouvait atteindre au moins 7 ans dans le SAC de Monte da Guia.

Cette étude démontre le potentiel de séries temporelles de photos-mosaïques sous-marines géo-référencées pour le suivi à long terme de populations de macro algues. Ce travail constitue également une base solide pour de futurs développements et applications à d'autres espèces, pouvant ainsi contribuer à une meilleure connaissance de l'écosystème benthique.

Jury members

Ulg Members

Prof. Jean-Marie Beckers (president)

Prof. Jean-Marie Bouquegneau (promotor)

Prof. Vincent Demoulin

Prof. Jean-Paul Donnay

Dr. Sylvie Gobert

External Members

Prof. Ricardo Serrão Santos (co-promotor)
Department of Oceanography and Fisheries (DOP)
University of the Azores, Portugal.

Prof. Alexandre Meinesz
Laboratoire « Environnement Marin Littoral EA « ECOMERS»
Université de Nice Sophia-Antipolis, France.

Dr. Frederik Leliaert
Phycology Research Group
University of Gent, Belgium.

Acknowledgments

The following institutions are thanked for funding: the Belgian - Fonds pour la Recherche en Industrie et en Agriculture - FRIA, for the PhD grant; the Belgian National Funds for Scientific Research (FNRS) and the Belgian French Community (Communauté Française) for travel grants; the Portuguese Fundation for Science and Technology (FCT) for providing a frame and logistical support for the present work within the context of the MAROV project (FCT - PDCTM/P/MAR/15249/1999).

I am specifically gratefull to Prof. Jean-Marie Bouquegneau (Laboratory of Oceanology - Uinversity of Liège) and Dr. Ricardo Serrão Santos (Department of Oceanography and Fisheries - University of the Azores) for giving me the possibility to realise this project, for welcoming me in their departments, for the orientation, trust, autonomy and everlasting support they offered me through everything.

I also thank most sincerely:

Sylvie Gobert, Frederico Cardigos and Fernando Tempera for supporting the idea and following the development of this project, for their multiple advises, sharing, and for keeping me on track in the most difficult moments.

Profs. J.M. Beckers and V. Demoulin for offering me the possibity to continue this work while learning more in their laboratories, for their numerous advises and mainly patience.

Prof. J. Nihoul and Marie-Laure Grégoire for the chance they gave me to join ULG and start learning ocean sciences within the Aral-Kum project, as the whole teams of the Laboratory of Oceanology and of the GeoHydrodynamics and Environmental Research departments.

Profs. A. Pascoal and J.P. Costeira (I.S.T., Portugal) for their hosting and enlightning orientation as support in exploring video mosaicking solutions.

Prof. J-P. Donnay (ULG, Belgium) for advises on statistical and geometric considerations.

Jean Marie-Théate for teaching me the first bubbles of SCUBA-diving.

Renzo Biondo for so many tecnical solutions and for the nutrient analysis, as also Norberto Serpa and Renato Bettencourt for their permanent logistial support at sea and for making me laugh so much with their true words: « O maluco belga que queres contar todas as algas do Faial » (Norberto); and the terrible one « Vocês cientistas, são todos eguais » (Renato).

All the volunteer divers and skippers involved in this research for their countless help to reach the study sites and for underwater work: David Abecassis, Sergio Bairos, Hugo Diogo, Rita Costa, Mirko de Girolamo, Rogério Ferraz, Pedro Frade, Eva Giacomello, Vera Guerreiro, Paulo Martins, Alexander Penas, Rui Prieto, Vítor Rosa, Vanessa Santos, Marco Aurelio, Teresa Dias, Alexander Penas, Catherine Jadot, Annick Donnay, Jaen Nieto Amat, and all the ones I miss to mention now. At their turn, day after day, they didn't hesitate to come to work and help me underwater, even in the middle of the winter waves. Grande abraço a todos!!

Wajih Youssef and Patsy Desclin for their collaboration and exchanges within the frame of their Master degree, as François Delobel for his encouraging help in some of the imagery processing.

Alain Losier, Antonio Pascoal, Thierry Van Brabant, Benoit Van Dieren, Anne Godertier, Jean-Claude Pierrot, André Masson and Isabelle Nuyts for sharing their various rich visions and changing mines.

Bauduin, Yukiko and Thomas Van Cauwenberghe, as Prof. Marie-France Destain, for trusting me.

Gaby Castillo for his various advises in the assistant work and Rosa Gago for her important support in final editing work.

Last but not least, I thank all my family and friends, particularly my parents Jacqueline Duhayon and Michel Sirjacobs, as my sister Laure, for their teaching, love, for being always there with complete support. My cousins Mecaël and David Yuan, Elisabeth and Michel for final readings, but also Philippe Deplechin and Armand Manenti for taking me early sailing at sea between storms.

Michal Shimoni for having encouraged my idea to move towards ocean sciences at ULG.

David Monticelli (the reviewer champion), Sophie Collette (another Alexandra David-Neel), Regis Paumen (the quietening strength), Franck Velay (the philosophical happiness center) and Géraud Devred (the humanist) for more than 18 years of indefectible friendship.

And above all I thank my son, Noam, for his incredible understanding, patience, surprising words, as for helping by cooking sometimes in the last weeks...

To all of you,

Thank you !
Merci!
Obrigado!

"The backside of the moon is better known than the 70 % of our planet that are covered by oceans. In order to meet the challenges of the next decade, major investments in marine science and technology are necessary. The future of the oceans is the future of mankind"
Peter Herzig - EurOCEAN 2010 Press release (10.09.2010).

General contents

Chapter Contents

Chapter 1 – Introduction

Chapter 2 - The environment of Monte da Guia SAC and selected study sites

Chapter 3 - The macroalga *Codium elisabethae*

Chapter 4 - The potential of visible remote sensing for benthic ecological studies

Chapter 5 - Benthic environmental conditions

Chapter 6 - *Codium elisabethae in situ* and laboratory studies

Chapter 7 - *Codium elisabethae* population structure and dynamics inferred by seabed visible imagery

Chapter 8 - General conclusions

Chapter 9 – Perspectives

10 – Annexes

List of illustrations and tables

Chapter 1:

Chapter 2:

Chapter 3:

Chapter 4:

Chapter 5:

Chapter 6:

Chapter 7:

Chapter 8:

Chapter 9:

Acronyms

ABISS	Automated Benthic Image-Scaling System
AUV	Autonomous Underwater Vehicle
AzC	Azores current
CASI	Compact Airborne Spectrographic Imager
CALD	site of Caldeinrinhas within Monte da Guia SAC, Faial, Azores.
C. elisabethae	*Codium elisabethae* O.C. Schmidt
C. bursa	*Codium bursa* C. Agardh
CCD	Charged Coupled Device
DOP	Departamento de Oceanografia e Pescas; Department of Oceanography and Fisheries - University of the Azores. http://www.dop.uac.pt/
DIVA	Data Interpolating Variational Analysis
GIS	Geographic Information System
GPS	Global Positioning System
LLSS	Laser Line Scan Systems
MAROV	Mapping of Marine Habitats of the Azores using Robotic Ocean-Vehicles. Research project funded by FCT, Fundation forSciences and Technology, Portugal.
MPA	Marine Protected Area
NOAA	National Oceanic and Atmospheric Administration, is a US federal agency focused on the condition of the oceans and the atmosphere.
NAC2	North Atlantic Current southern branch
NATURA 2000	European wide network of nature protection areas established under the 1992 Habitats Directive
PAR	Photosynthetic Active Radiation
PF	site of Ponta Furada within Monte da Guia SAC, Faial, Azores.
ROV	Remote Operated Vehicle
SAC	Special Area of Conservation (NATURA 2000 Network)
SACFOR	Scale of abundance : Abundant, Common, Frequent, Occasionnal, Rare
SCI	Site of Community Importance (NATURA 2000 Network)
SCUBA	Self Contained Underwater Breathing Apparatus
SPA	Special Protected Area
STARESO	Station de Recherches Océanographiques et Sous-marines http://www.stareso.ulg.ac.be/Stareso/Stareso.html

1 - Introduction

Table of contents

1.1 Specific objectives and document structure

Within the context described in section 1.2 and 1.3, the present research aims at producing a precise description of the population static parameters (size histograms, density, cover rate) and dynamic parameters (growth rate, primary production) of the macroalga *Codium elisabethae* occupying the Special Area of Conservation of Monte da Guia, Faial, Açores. This first precise quantitative evaluation of the *Codium elisabethae* life cycle, population dynamics and internal nutrient concentration is an important step in the evaluation of the ecological role of the species within this highly valued ecosystem, as toward the potential exploitation of *Codium elisabethae* as a bioindicator of coastal environmental changes. It is our hope that the present thesis will highlight the benefit and efficiency resulting from the use of an underwater image mosaicking approach for long term and spatially precise monitoring of benthic populations, thereby archiving a high level of spatial organizational information. This work will also propose explanations on how the local benthic habitat specificities (topography and induced hydrodynamic) do shape *Codium elisabethae* population dynamics and reproduction.

The specific objectives of the present thesis are:

- to describe the physical dynamic of 2 benthic habitat sites occupied by contrasting structures of populations of *C. elisabethae* within Monte da Guia SAC.
- to confirm and quantify the nutrient enrichment of the internal seawater of *C. elisabethae*, as was demonstrated earlier for *C. bursa*.
- to establish the seasonal dynamics of the sexual and vegetative reproduction of *C. elisabethae*, and demonstrate the possible summer fertility.
- to quantify the fluctuations of density, biomass, population structure, growth rate, primary production and lifespan of the studied *C. elisabethae* populations over a 2 year period, based on the analysis of seabed underwater imagery.
- to analyse the possible links between the various information produced (physical habitat and status-environment-reproduction-population dynamic)
- to conclude on: 1) the new information produced in regard to pre-existing knowledge on *C. elisabethae* and *C.bursa*; 2) their consequences on the potential exploitations of *C. elisabethae* and *C. bursa* as complementary bioindicators of environmental changes over the Macaronesian, Mediterranean, and European Atlantic coasts; 3) the high potential of underwater imagery as an efficient tool for expanding the possibilities of benthic ecology studies and monitoring over large spatio-temporal scales.

The structure of the document (Fig. 1) can be outlined as follows.

The general and specific contexts of the thesis are given in complement to the present introduction.

The first part consists of several literature reviews of the main topics addressed in the thesis. Chapter 2 gives a general description of the environment of the Monte da Guia SAC and of the selected study sites of Caldeirinhas and Ponta Furada located within the southern coast of Faial. A synthesis of the current knowledge of the macroalga *C. elisabethae* is provided in chapter 3, together with some general considerations about the *Codium* genus and useful comparisons with the sister species *C. bursa*. Chapter 4 underlines the requirements of new

spatial approaches in benthic ecology and the potential of visible imagery for benthic ecology in general and for seaweed ecology in particular.

The second part presents the methods, results, and discussions of the thesis. Chapter 5 gives a synthetic characterization of the environmental conditions existing within the two benthic habitat studied (temperature, currents, photosynthetic active radiation, turbidity, nutrients), based on the analysis of *in situ* water samples and of data sets produced by a battery of sensors deployed *in situ* during a complete year. Chapter 6 describes the results of *in situ* and laboratory measurements of the macroalga morphology, population structure, reproduction dynamics, and nutrient concentrations within the algae's lumen (enclosed seawater volume). Quantification of population dynamics parameters, growth dynamics and biomass production are detailed in Chapter 7 together with the developed methodologies of submarine image acquisition and exploitation.

A third part consists of general discussions, conclusions and perspectives. A general conclusion (chapter 8) summarizes novel information produced, discusses and emphasizes interesting links between results and finally the main perspectives are summarized in chapter 9, focusing on the important points that need to be addressed to expand the limits of our knowledge concerning *Codium elisabethae* and the studied benthic habitats, as concerning future improvements and potential applications of the imagery method developed.

Chapter 1 - Introduction

Bibliographic research

Chapter 2 - The environment of Monte da Guia SAC and selected study sites
Chapter 3 – The macroalga *Codium elisabethae*
Chapter 4 - The potential of visible remote sensing for benthic ecological studies

Material, methods, results and discussions

Chapter 5 - Benthic environmental conditions
Chapter 6 - *Codium elisabethae* in situ and laboratory studies
Chapter 7 - *Codium elisabethae* population structure and dynamics inferred by seabed visible imagery

Synthesis

Chapter 8 - General Conclusions
Chapter 9 - Perspectives

****

Chapter 10 - Annexes

Figure 1 - Structure of the Thesis.

1.2 General context

1.2.1 Importance of seabed habitats

1.2.1.1 Coastal seabed habitats

The oceans harbor a great diversity of organisms whose distribution and ecological preferences are often poorly understood (Tyberghein *et al.*, 2011).

The notion of "Coastal Seabed Habitat" is very broad. It defines both "**what is where**" in terms of **mineral and biological resources** of the marine coastal zone, and "**what happens where**" in terms of **environmental constraints** on the living and geological processes. In this sense, "habitat" is not only the substrate and shelters encountered by the "inhabitants", but also the local dynamic "aquatic weather" they do benefit from and have to cope with. These notions are of high importance in the sense that they will define **how productive is a specific area** regarding particular primary producers, or how much important are small **localized zones where conditions are favorable for the reproduction** of other species which, at later life stages, disperse on the whole coastal zone we are interested about.

Biological communities are changing drastically in response to global climate change, changes in use by human populations, and invasive species. To study the impact of such changes on the marine environment, biologists require a detailed understanding of the diversity and distributions of marine organisms (Wiley *et al.*, 2003). It is due to the integration of substrate, environmental dynamics, and biological communities notions that **some benthic habitat can be understood as biodiversity hotspots (Ready *et al.*, 2010) or refuges in face of anthropic pressures (Halpern *et al.*, 2008), global climate changes (Thomas *et al.*, 2004), or introduction of exotic species (Thuiller *et al.*, 2005; Cardigos *et al.*, 2006)**. **Such types of areas should certainly be given a specific attention (protection) for the establishment of coastal marine management plans** supposed to protect biodiversity as well as to optimize present and future exploitability of resources. Monitoring of key population dynamics, species distribution modelling (Tempera, 2009; Tyberghein *et al.*, 2011) and ecosystem modelling will increasingly contribute to improve ecological knowledge and inform marine ecosystem management and conservation.

1.2.1.2 Importance of seabed habitats ecosystem services

The seabed is the most extensive habitat on the planet. It occupies at least 75% of the Earth's surface, far more if the planar areas of the shelves and slopes are taken into account. It follows that fluxes of materials across the sediment–water interface, and the mechanisms that mediate and constrain those fluxes are likely to have global significance (Raffaelli *et al.*, 2003). The global importance of seabed has been evaluated for nutrient cycling, which for marine shallow water systems is superior to 40,000 US$/(ha.year), representing 1.86% of the value of this service for all terrestrial and aquatic systems (Constanza *et al.*, 1997, in Raffaelli *et al.*, 2003). Benthic ecosystem equilibriums are known to have a very important impact on the bentho-pelagic coastal fisheries production as their dynamics are closely interrelated.

Marine biodiversity is higher in benthic than in pelagic systems, and in coasts rather than the open ocean since there is a greater range of habitats near the coast (Gray, 1997). Losses of marine diversity are highest in coastal areas largely as a result of conflicting uses of coastal habitats. According to Gray (1997), the best way to conserve marine diversity is to conserve habitat and landscape diversity in the coastal area. This author suggests that marine protected areas (MPAs) are only a part of the conservation strategy needed and that the framework for coastal conservation is an integrated coastal area management where one of the primary goals is the sustainable use of coastal biodiversity.

The biodiversity is a sensitive key factor in the ecosystem services, in terrestrial (Hector and Bagchi, 2007) as in aquatic environments (Duarte, 2000; Schaffelke *et al.*, 2006). Field studies of biodiversity fluctuations do require habitat structure information and will certainly increasingly require integrating a spatial ecology dynamic approach for any modelling attempt to answer practical management questions concerning natural resources. Indeed, Bulling *et al.* (2006) consider that the integration of spatial scales is one of the future challenges to allow ecosystem models to address the biodiversity–ecosystem functioning. However, they underlie that the first and greatest limitation in this progress is due to our lack of knowledge of the real scaling relationships that drive ecosystem dynamics, which confirms well the imperative need to develop new methods to explore seabed habitat structures and monitor their changes at larger spatio-temporal extents and with enhanced resolutions.

1.2.1.3 Increasing damages to benthos ecosystems

The example of the French Mediterranean shore. The marine littoral of the Region "Provence-Alpes-Côte d'Azur" suffered during a long period of multiple degradations (Meinesz *et al.*, 1981, 1982 and 1985) of which mainly (i) irreversible destruction of shallow habitats (0-20 m) by artificial land reclamation over the sea, (ii) general degradation of ecosystems due to non-treated sewage outfalls and to the increasing turbidity of coastal waters and (iii) increasing professional and recreational overfishing.

Trawling impact. Among the problems affecting benthos ecosystems, Schmidt (1998) as well as Malakoff (1998) summarized important researcher's interrogations and progresses concerning the impact of trawling on seabed. Particularly, Watling and Norse (1998) compared the habitat damage caused by trawling with forest clear-cutting (long denounced by biologists as a major threat to biodiversity). Thrush and Dayton (2002) underlined the importance of being able to consider correctly the spatio-temporal scales of disturbances in regard to the extent of habitat and its recovery time.

Decline of coral reef communities. Similarly, the recent decline in the condition of coral reef communities worldwide has fuelled the need to develop innovative assessment tools to document coral abundance and distribution rapidly and effectively. While most monitoring programs rely primarily on data collected *in situ* by trained divers, digital photographs and video are used increasingly to extract ecological indicators, to provide a permanent visual record of reef condition, and to reduce the time that divers spend underwater (Lirman *et al.*, 2007).

The importance of ecologically sensitive, degraded and over-exploited seabed ecosystems raises the need to monitor them frequently, over a large scale, and in a cost-efficient and spatially explicit manner.

1.2.2 Benthic ecology dynamics requires spatial considerations

Coastal biodiversity and biological resources management depend on a fundamental knowledge concerning habitats as distribution and dynamics of local populations and metapopulations (Kritzer and Sale, 2006). Their maintenance and variations are the results of complex interactions between the degree of connectivity of populations within their biogeographic range and the dynamics of forcing factors including changes in environmental and anthropic pressures (Kritzer and Sale, 2006). Greatly complex questions such as the process of designing marine protected areas for biodiversity or stock protection, or the estimation of the level of sustainable exploitability of benthic marine biological resources require complex spatialized considerations (seabed habitat maps, seabed habitat hydrodynamics, local population dynamic, genetic proximity between populations).

The whole coastal exploitation should be thought in the long term as an insertion, a fusion in the dynamics of natural ecological processes in order to be sustainable. Long term data acquisition and monitoring of coastal marine habitat status are required to confirm the potential consequences of the various coastal uses on specific changes of the environmental status and of economic productivities. It is only after a good knowledge of the distribution and dynamics of coastal marine life is acquired that an integrative analysis of economic and environmental pressures affecting natural processes will become possible. It is on the basis of such integrated understanding and with growing experience in each particular type of coastal areas that the definition of sound management plans will gain in efficiency.

In order to link realistically and at a large scale the marine habitat status with notions of sustainable development of marine coastal uses, new extensive surveying approaches are required in complement with the traditional approaches developed in localized benthic research. However, in the coastal environment, precise habitat description and population monitoring are particularly difficult to achieve. New emerging techniques are increasingly needed to allow more extensive, frequent, and precise monitoring. These techniques include processing of images (visible or acoustic) obtained from platform ranging from satellites in space down to underwater automated robots or handled in scuba-diving, as well as the permanent installation of seabed sensors. Still to be combined with classical field work monitoring, sampling and laboratory studies, such information can be integrated into spatialized environmental modelling

Extensive, precise and frequent seabed surveying constitutes an expensive monitoring work but is the price to pay to allow a rational management of resources without taking the risk of damaging them further. Yet, modern technology is progressively reducing these monitoring costs by expanding our abilities in term of imaging resolution, navigation automation and range, and subsequently in term of spatio-temporal coverage efficiency.

Modern seabed mapping becomes thus a basic requirement for the general study of marine ecosystem processes and changes, the implementation and long term monitoring of Marine Protected Areas (MPAs), the management of marine resources, and the monitoring and modelling of species' distributional shifts.

Thus, the optimization and sustainable management of coastal uses require precise and frequent evaluation of the whole coastal habitats status, or at least of some well-designed sub sampled particular areas, chosen as indicators of the status of exploited zones and of protected areas.

1.2.3 Importance of seaweed communities

Seaweeds can grow as individuals, but they more frequently live together in communities with other seaweed and animal species. Seaweed communities affect and are affected by the environment, and are among the most productive marine plant communities on earth (Leliaert and Coppejans, 2004). Together the intertidal and subtidal zones give rise to a narrow coastal area that accounts for less than one per cent of the Earth's surface (Leliaert and Coppejans, 2004). However the productivity of this region can equal or exceed that of most terrestrial communities (Dawes, 1998).

In many coastal ecosystems, seaweeds play an important contribution: as primary producers feeding the basis of trophic chains (herbivorus and detritivorus), as contributors to the nutrient cycling (Alvera-Azcárate *et al.*, 2003; Lepoint *et al.*, 2004 and 2007), as sheltering and supports to other species. In their review on marine autotrophic production, Duarte and Cebrián (1996) produced general estimations of the fate of Net Primary Production (NPP) of macroalgae as follows. Concerning the local ecosystem trophic chain, at least 40 % of macroalgae NPP feed the local decomposition chain, while around 34 % is consumed by the herbivores. The NPP part which is not useful to the local ecosystem is estimated at 24 % (and up to 44%) for the exportation by advection out of the system, and from 0.4 till 6 % for the exportation by storage in sediments.

Within benthic ecosystems, seaweed communities are an important component and indicator of the coastal benthic equilibriums, and deserve as such a detailed attention.

As a consequence, an increasing number of coastal environmental monitoring programs include an estimation of seabed primary producers, and macroalgae in particular. For instance, after underlining that coral reef monitoring programs often focus narrowly on hard corals despite the low coral abundance but high macroalga abundance on many coral reefs worldwide, Miller *et al.* (2003) advocated the inclusion and standardization of the estimation of reef macroalga abundance as a contribution to improve and coordinate coral reef monitoring at a large scale.

1.3 Specific context of the thesis

1.3.1 The environment and European conservation status

Natura 2000 is a European ecological network composed of sites designated under the Birds Directive (Special Protection Areas, SPAs) and the Habitats Directive (Sites of Community Importance, SCIs, and Special Areas of Conservation, SACs). It aims at preserving biodiversity by conserving natural habitats, wild flora and fauna in the European Union territory. It is composed of sites classified as Special Protection Areas (SPA), by the Council Directive 79/409/EEC of 2 April 1979 on the conservation of wild birds, including the migratory species and the habitats where they occur. A Site of Community Importance (SCI) is defined in the European Commission Habitats Directive (92/43/EEC) as a site which, in the biogeographical region or regions to which it belongs, contributes significantly to the

maintenance or restoration at a favorable conservation status of a natural habitat type or of a species and may also contribute significantly to the coherence of Natura 2000, and/or contributes significantly to the maintenance of biological diversity within the biogeographic region or regions concerned (European Environment Agency, 2010). The management of such areas must be ecologically, economically and socially sustainable. In the same Natura 200 context, the status of "Special Area of Conservation" (SAC) can be attributed to some of these SCI, according to the decision taken by environmental authorities to establish management priorities and necessary conservation measures for such particularly important sites.

The Macaronesian region includes five mid-Atlantic archipelagos (Azores, Madeira, Selvagems, Canaries, and Cape Verde; Fig. 2) and is traditionally recognized as biogeographic unit based on the hypothesis that the terrestrial flora is a relic of a formerly broadly distributed subtropical Tertiary flora, although this concept is being refined based on distinct affinities between islands and with neighboring continental Africa according to plant groups (Vanderpoorten *et al.*, 2007). All of these islands are of volcanic origin and have never formed part of a continent. The contrasting landscapes and the mild climatic conditions have created an ideal environment for a particularly rich array of species and habitats. Despite representing only 0.3 % of the EU territory, the Macaronesian region hosts no less than 19 % of the habitat types listed in the Annex I of the Habitats Directive (European Commission, 1992) and 28 % of plants listed in Annex II of that document. The rate of plant endemism is exceptionally high, the islands being in fact amongst the top places in the world for plant biodiversity (ICNB, 2011).

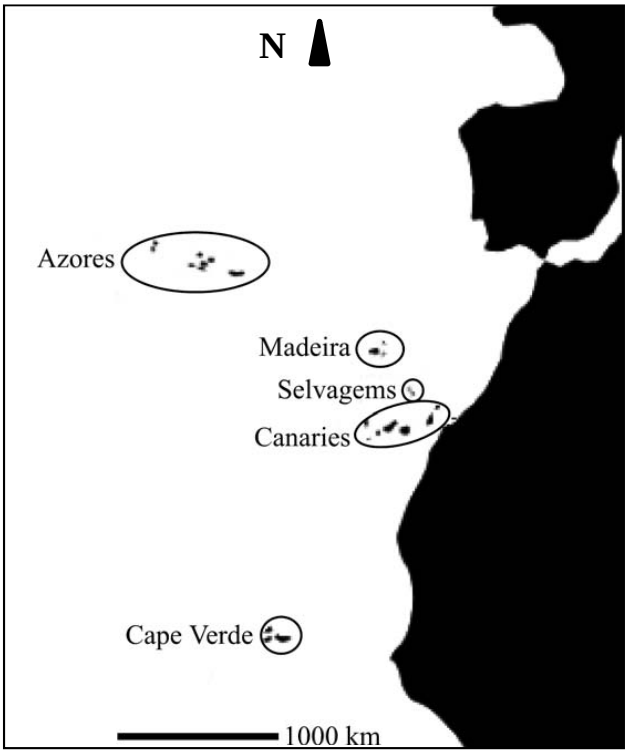


Figure 2 - Map of the biogeographical region of Macaronesia (adapted from Vanderpoorten *et al.*, 2007).

The Azores archipelago consists of nine islands stretched along 600 km of the northern part of the Mid Atlantic Ridge (Fig. 3), a third of the way between the Iberian Peninsula and Newfoundland in Canada. At a distance of about 1500 km from mainland Portugal, the archipelago forms one of the two Autonomous Region of the Portugal (the other being the archipelago of Madeira), and its wide extension results in an exclusive economic zone of nearly 1.1 million km2. The Azorean climate is mild and wet, with considerable precipitation during the summer (Silva, 2001). The oceanic influence stabilizes the air temperature with an annual mean of 17.3 ºC and an average relative humidity of 84% (Silva, 2001). Overall, the weather and structure of the Azores natural terrestrial ecosystems match those of a sub-tropical environment (Haggar, 1988), but they have a different species composition than the other Macaronesian archipelagos, being more heavily influenced by Northern European rather than Mediterranean species. The Azorean islands also have large parts of relatively gentle topography with undulating hills and peaks compared to the very abrupt precipices and accentuate topography of the Madeira archipelago. This makes them ideal for dairy farming.

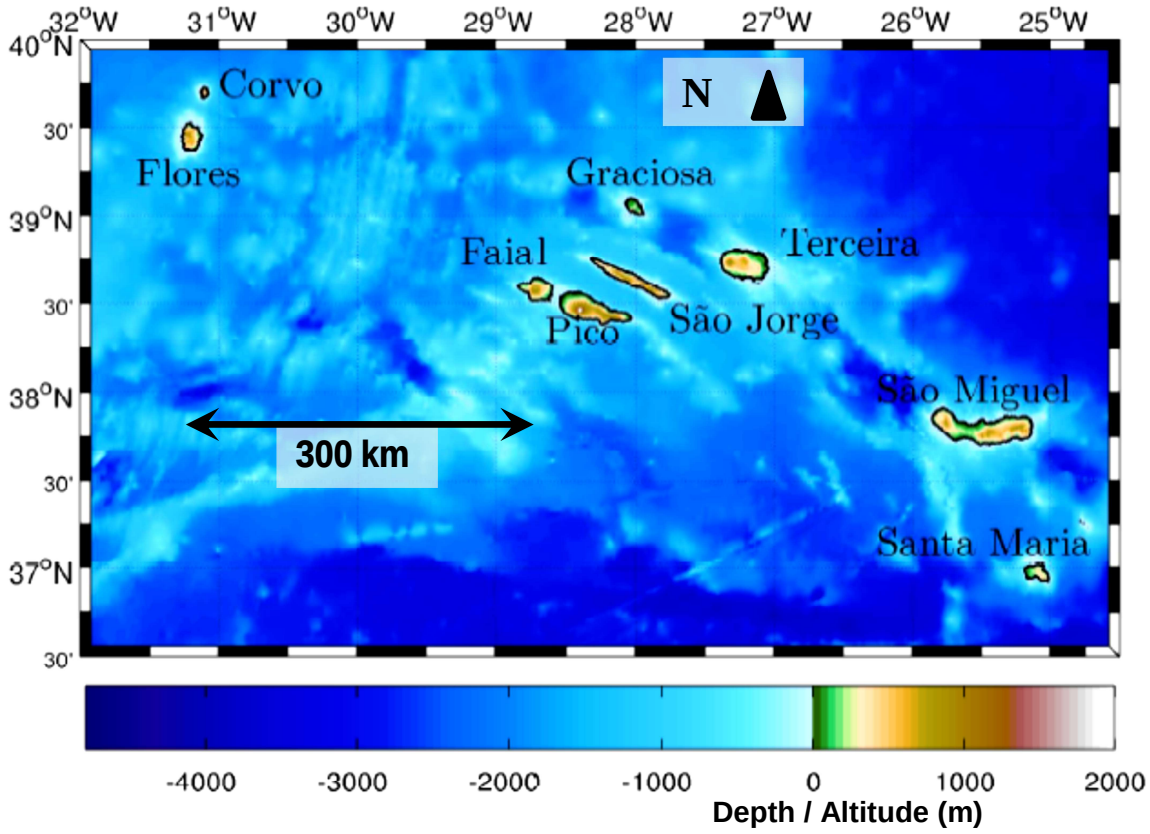


Figure 3 -Map of the Azores archipelago (topography and bathymetry in meters).

Hard seafloor habitats (also known as reefs) concentrate most of the conspicuous marine biodiversity and of the demersal fishing effort in the Azores (Seabra *et al.*, 2005). Among the species that directly depend on them for either the whole or part of their life cycles are a variety of benthic macroalgae, invertebrates and demersal fish that are important both for economic and/or ecological reasons (Seabra *et al.*, 2005).

There are 23 areas classified as Sites of Community Importance in the Azores, among which 17 are marine sites (European Commission, 2009). Based on early reviews (Santos *et al.*, 1995), IMAR-DOP/UAç put forward projects to develop management schemes for six of them. Each of these sites has special features that have contributed for their selection: isolation and wilderness (Formigas bank), particular social context (Corvo Island), unique environment (D. João de Castro bank) and proximity to research facilities (Monte da Guia, Baixa do Sul and Madalena islets). Along with a series of other Azorean sites, the ones mentioned above are now designated as BIOMARE Sites (Cardigos and Tempera, 2002). The present research is carried out in the SAC of Monte da Guia, which occupies the southeastern coast of Faial island.

1.3.2 Importance of *Codium elisabethae*

Codium elisabethae O.C. Schmidt (1929) is a dark green spongy balloon-like species (Fig. 4). The perennial thallus is formed of a spherical entangled network of siphonous filaments enclosing an isolated seawater volume.

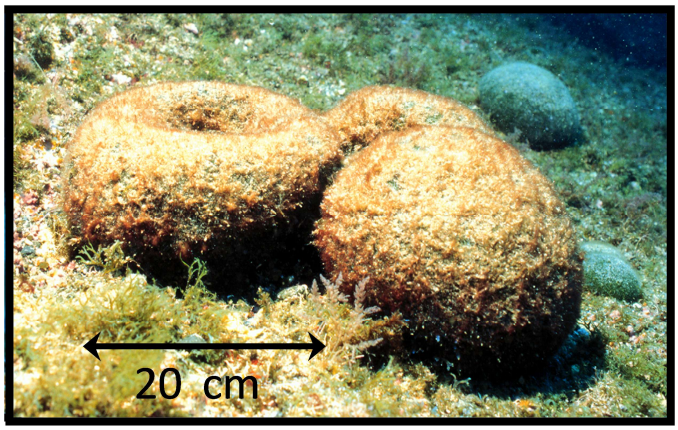


Figure 4 - *Codium elisabethae* individuals from the Azores (© ImagDOP/Universidade dos Açores)

Most of the previous work achieved on *Codium elisabethae* concerns mainly morphological descriptions. Only rare studies have focused either on an evaluation of *Codium elisabethae* habitat (Tempera, 2009) or on a description of its densities (Neto, 1997 and 2000). This last work exploited only relatively small samples observed randomly each season, and did not address reproduction, growth rate and primary production (Neto, 1997). However, precise measures of biomass, primary production, and impacts on the benthic nutrient storage and cycling are required steps to evaluate the ecological importance of *Codium elisabethae* populations.

Considering its wide distribution and important local densities (ranging in many places from important to dominant according to SACFOR scales (Tempera *et al.*, 2001), the macroalga *Codium elisabethae* occupies an important ecological place within algal communities of the SAC of Monte da Guia.

1.3.3 Potential of macroalgae as bioindicators

In the Mediterranean Sea, species from the genus *Cystoseira* (Fucales, Sargassaceae) are structuring the rocky habitats in three dimensions, hosting under their canopies a high biodiversity, and are one good example of the potential role of **long living sessile organisms as permanent sensitive integrating detectors of environmental changes**. Many authors are reporting a decline of these species in many parts of the Mediterranean Sea (Janssens *et al.*, 1993, Munda, 1993). Following the monitoring of *Cystoseira* populations on the continental French coasts in 2007-2008 and the analysis of the cause of their regression in the Mediterranean basin, Thibaut *et al.* (2009) suggested that habitat destruction and overgrazing by herbivores are the main cause of massive regression for shallow waters species, while mussel farming are responsible for the decline of *Cystoseira amentacea var. stricta* and *Cystoseira mediterranea*. These observations are not consistent with the studies by Munda (1993) for the Adriatic and by Janssens *et al.*, (1993) and Demoulin (personal comunication) for Corsica, clearly implying eutrophication as cause of degradation for *Cystoseira* populations.

Numerous other benthic organism populations (mainly macroalgae) might be used to reveal the importance and trends in environmental changes, -even if using closely related species for different regions-, providing common control parameter can be tuned to local conditions.

The case of *Codium elisabethae* and *Codium bursa*

Codium elisabethae O.C. Schmidt (*C. elisabethae*) and *Codium bursa* C. Agardh (*C. bursa*) are very similar species known up to now to differ only by small microscopic morphological differences in the shape and size of their utricles (being mucronate for *C. elisabethae* and roundish for *C. bursa*), and by their geographical ranges. Relatively little is known on the Macaronesian endemic *C. elisabethae* as compared to *C. bursa*, whose distribution range covers West-European, North-Western African and the Mediterranean coast.

Codium bursa was identified as a potentially good indicator species of coastal environmental changes (Duarte *et al.*, 1999) as it is one of the few known long living sessile benthic organisms (together with the seagrass *Posidonia oceanica* and the bivalve *Pinna nobilis*). Further, it's interesting adaptation to oligotrophy by environmental nutrient concentration, storage and recycling in its internal seawater volume are raising interest on its sensitivity to environmental changes.

Considering the known similarities between *C. bursa* and *C. elisabethae*, it can be hypothesized that *C. elisabethae* would constitute an interesting complementary bioindicator of environmental changes if its growth and reproduction dynamics, its long-living characteristic, as well as its adaptation to oligotrophy by environmental nutrient concentration could be demonstrated. This could expand the geographic area (Mediterranean, European, North African Atlantic coasts, and the whole Macaronesia coasts) for which similar organisms could be exploited for coherent environmental monitoring. Furthermore, we hypothesize that there might be some interesting environmental 'bioindicating' parameters to read in the trends of changes of the population structure of *C. elisabethae*, providing that 1) they are extracted from populations which are permanent at decadal or quinquenal scale and not transient (colonizing populations, occupying extreme edge of habitat condition ranges), and 2) they are sampled from sufficiently large population in permanent study zone so as to produce robust

statistics, reducing the bias resulting from the effect of small scale population patchiness and of variable random distribution used by each repetition of traditional *in situ* subsampling approaches.

Learning about the ecology of a potential bioindicator.
Scuba diving explorations at *C. elisabethae* maximal density depth range (20-25m) along the coasts of Faial and Pico revealed contrasting populations stands of *C. elisabethae* according to local environmental conditions. This raises numerous interesting questions about the auto-ecology of these algae and about their interaction with their habitat. What elements of the benthic habitat environment and the life cycle dynamics do structure these local populations? How stable in time are these local populations? What is the range of their primary production contribution to the benthic trophic chains? These questions are first addressed by the present research as they will open the way to long term monitoring interests. Furthermore, we can ask longer term questions: "Can some changes in the population parameters of these long living benthic organisms be exploited as integrating indicators of environmental changes? Can some of these populations be considered as robust and stable sources and others just as sensitive transitory colonizers?"

Verifying basic interesting properties of a potential bioindicator.
Environmental bioindicating proxies should be precisely searched and defined by future researches, but such work can only be envisioning the common exploitation of *C. elisabethae* and *C. bursa* providing similar interesting properties (long living; nutrient concentration within lumen water / nutrient limitations / growth rates) could be confirmed or quantified for both species, and providing subsequent efficient and robust monitoring methods can be established.

1.3.4 Monitoring of Natura 2000 sites within MAROV project

The Department of Oceanography and Fisheries of the University of the Azores (DOP/UAç) devoted much work since 1976 in order to understand the processes that control the marine ecosystems around the islands, and to develop integrated monitoring schemes of the coastal biodiversity and resources (Cardigos and Tempera, 2002). Considering the importance of the reef habitats, DOP/UAç has concentrated a large segment of its research activities on hard bottom species, assemblages or biotopes. The knowledge acquired has been relevant not only for scientists but also for managers and decision-makers (Seabra *et al.*, 2005). Concrete consequences ranged from the rational scientific selection of several sites of particular ecological or economical interest, to the legal implementation of adapted monitoring or protection status (Natura 2000 Sites of Community Importance, Marine Special Areas of Conservation, integral reserves), till the development of management schemes for several of these sites. A long term follow up mapping of these reef habitats and study of associated species are however required to allow enhanced management measures and more accurate designs for the areas aiming at protecting biodiversity and essential habitats for species of commercial and touristic interests (Seabra *et al.*, 2005). In this context several projects of the DOP are oriented towards the ecological evaluation of the benthos through mapping approaches, as well as towards the development of new methodologies and technologies for such monitoring.

The present research was initiated in the context of the MAROV project (Mapping of Marine Habitats of the Azores using Robotic Ocean Vehicles; Pascoal, 2011 and Santos, 2011). MAROV produced numerous marine habitat and biodiversity mapping applications for marine SCIs and SACs in the Azores (Santos *et al.*, 2001a and 2001b; Cardigos and Tempera, 2002; Tempera *et al.*, 2004; Seabra *et al.*, 2005; Tempera and Santos, 2006) with the objective of deriving guidelines for their management. The project aimed also at determining habitat attributes that significantly affect the distribution and dynamics of harvested species.

Within this context, the potential of underwater visible imagery is widely explored, not only for the study of presence and spatial distribution of benthic species, but also for the quantification of populations structures and dynamic of *Codium elisabethae* macroalga populations within the site of Monte da Guia (Faial), as listed in the 2005 report of the Working Group on Habitat Mapping (WGHM), International Council for the Exploration of the Sea (ICES, 2005). The site of Monte da Guia was long referred to as SCI but it was recently defined as a Special Area of Conservation (SAC) by the environmental authorities.

1.3.5 Importance of underwater visible imagery for seaweed monitoring

Historically, advances in knowledge of benthic community structure and functioning have necessarily relied upon destructive sampling devices (grabs, cores, anchor dredges, etc.) that lose valuable contextual information in the process of sampling (Solan *et al.*, 2003). They specify that among the vast array of instrumentation devices capable of measuring benthic dynamic processes both acoustic and optical imaging devices have already played a significant role and should be further developed to facilitate the collection of ecological information.

After presenting the importance of habitat mapping (Greene, 2007) as an introduction to the final MESH project Conference "Mapping European Seabed Habitats, a framework to support Sustainable Management", Professor Gary Greene formulated the conference concluding remarks. As such, he stressed that habitat is not limited to the physiographic aspects and identified the challenging need to superimpose two types of information to currently available topographic and geological habitat maps.

The first need is the production of precise, continuous and extensive biological occupation maps through the development of underwater visible remote sensing methodologies.

The second need is a precise description of the benthic seasonal environmental conditions encountered in the habitats (hydrodynamics, temperature, light), which should be progressively extended into spatialized information by combining local benthic *in situ* measurements with the modelling of precise physiographic maps produced with acoustics.

Pauly and De Clerck (2010) underlined a third challenge: by comparison with coral reefs and seagrass meadows which are known to form large and homogeneous assemblages, seaweeds communities are spectrally and spatially very heterogeneous. This difficulty is challenging for above surface remote sensing studies and constitute one argument for the development of underwater remote sensing applications as this latter technique can potentially collect far more precise spectral and organizational information at small scales and is adapted for deeper applications (below 5-10 meters and down to thousands of meters).

Some elements of these three last challenges are addressed by the present research.

1.4 References

Alvera-Azcárate, A., Ferreira, J.G. and Nunes, J.P., 2003. Modelling eutrophication in mesotidal and macrotidal estuaries. The role of intertidal seaweeds. Estuarine, Coastal and Shelf Science 57: 715-724.

Bulling, M. T., White, P.C.L., Raffaelli, D., Pierce, G J., 2006. Using model systems to address the biodiversity–ecosystem functioning process. Marine Ecology Progress Series, 311: 295-309.

Cardigos, F. and Tempera, F., 2002. Mapping of marine biodiversity at marine special areas of conservation (SACs) in the Azores. implementation of large-scale long-term marine bio diversity research in Europe. NEWSLETTER 3.
http://www.biomareweb.org/3.5.html

Cardigos, F., Tempera, F., Ávila, S., Gonçalves, J., Colaço, A. and Santos, R.S., 2006. Non-indigenous marine species of the Azores. Helgoland Marine Research, 60(2): 160-169.

Constanza, R., d'Arge, R., de Groot, R., Fraber, S., Grasso, M., Hannon, B., Limburg, K., Naeem, S., O'Neill, R.V., Paruelo, J., Raskin, R.G., Sutton, P. and van den Belt, M., 1997. The value of the world's ecosystem services and natural capital. Nature, 387: 253-259.

Dawes, C.J., 1998. Marine Botany. 2nd Ed. John Wiley and Sons, Inc. New York. 48 pp.

Duarte, C.M. and Cebrián, J., 1996. The fate of marine autotrophic production. Limnology and Oceanography, 41 (8): 1758-1766.

Duarte, C.M., Agustí, S., Kennedy, H. and Vaqué, D., 1999. The Mediterranean climate as a template for the Mediterranean marine ecosystem: the example of the NE Spanish littoral. Progress in Oceanography, 44: 245-270.

Duarte, C.M., 2000. Marine biodiversity and ecosystem services: an elusive link. Journal of Experimental Marine Biology and Ecology, 250 (1-2): 117-131.

European Commission, 1992. Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora.
http://ec.europa.eu/environment/nature/legislation/habitatsdirective/index_en.htm,
accessed 15/01/2011.

European Commission, 2009. Natura 2000 in the Macaronesian Region. European Commission, Environment Directorate General, Author: Kerstin Sundseth, Ecosystems LTD, Brussels.12 pp.
doi: 10.2779/81690
http://ec.europa.eu/environment/nature/info/pubs/docs/biogeos/Macaronesian.pdf,
accessed 12/01/2011.

European Environment Agency, 2010. Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora (art 1 k).
http://glossary.eea.europa.eu/EEAGlossary/S/site_of_Community_importance,
accessed 14/12/2010.

Gray, J.S., 1997. Marine biodiversity: patterns, threats and conservation needs. Biodiversity and Conservation, 6: 153-175.

Greene, G., 2007. The importance of habitats mapping. MESH final Conference: "Mapping European Seabed Habitats, a framework to support Sustainable Management", 14-15/3/2007, Dublin Castle, Dublin, Ireland.
http://www.searchmesh.net/pdf/Gary_Greene.pdf

Haggar, J.P., 1988. The structure, composition and status of the cloud forests of Pico Island in the Azores. Biological Conservation, 46: 7-22.

Halpern, B.S., Walbridge, S., Selkoe, K.A., Kappel, C.V., Micheli, F., D'agrosa, C., Bruno, J.F., Casey, K.S., Ebert, C., Fox, H.E., Fujita, R., Heinemann, D., Lenihan, H.S., Madin, E.M.P., Perry, M.T., Selig, E.R., Spalding, M., Steneck, R. and Watson, R., 2008. A global map of human impact on marine ecosystems. Science, 319: 948-952.
doi:10.1126/science.1149345

Hector, A and Bagchi, R., 2007. Biodiversity and ecosystem multifunctionality. Nature, 448(7150): 188-90.
doi:10.1038/nature05947
http://www.nature.com/nature/journal/v448/n7150/abs/nature05947.html

ICES. 2005. Report of the Working Group on Habitat Mapping (WGHM), 5–8 April, Bremerhaven, Germany. ICES CM 2005/E:05, 87 pp.

ICNB (Nature Conservation and Biodiversity Institute), Portuguese Ministry for the Environment, 2011.
http://portal.icnb.pt/ICNPortal/vEN2007/O+ICNB/Rede+Natura+2000/, accessed 15/06/2011.

Janssens, M., Hoffmann, L. and Demoulin, V., 1993. Cartographie des macroalgues dans la région de Calvi (Corse) : comparaison après 12 ans (1978-79, 1990-91). Lejeunia, 141: 1-62.

Kritzer, J. P. and Sale, P. F., 2006. Marine metapopulations. Ed. Kritzer and Sale, Elsevier Academic Press, Oxford, UK. 544 pp.

Leliaert F. and Coppejans E., 2004. Seagrasses and Seaweeds. Standard Survey Methods for Key Habitats and Key Species in the Red Sea and Gulf of Aden. PERSGA Technical Series No. 10. PERSGA, Jeddah. pp. 101-124.

Lepoint, G., Gobert, S., Dauby, P. and Bouquegneau, J-M., 2004. Contributions of benthic and planktonic primary producers to nitrate and ammonium uptake fluxes in a nutrient-poor shallow coastal area (Corsica, NW Mediterranean). Journal of Experimental Marine Biology and Ecology, 302(1): 107-122.

Lepoint, G., Jacquemart, J., Bouquegneau, J-M., Demoulin, V. and Gobert, S., 2007. Field measurements of inorganic nitrogen uptake by epiflora components of the seagrass *Posidonia oceanica* (Monocotyledons, *Posidoniaceae*). Journal of Phycology, 43(2): 208-218.
http://hdl.handle.net/2268/2484

Lirman, D., Gracias, N., Gintert, B., Gleason, A., Reid, R., Negahdaripour, S. and Kramer, P., 2007. Development and application of a video-mosaic survey technology to document the status of coral reef communities. Environmental Monitoring and Assessment, 125: 59–73
doi: 10.1007/s10661-006-9239-0

Malakoff, D., 1998. Papers posit grave impact of trawling. Science 18(282-5397): 2168.
doi: 10.1126/science.282.5397.2168b

Meinesz, A., Astier, J.M. and Lefèvre, J.R., 1981. Impact de l'aménagement du domaine maritime sur l'étage infralittoral du Var, France (Méditerranée occidentale). Annales de l'Institut Océanographique, Paris, France, 57(2): 65-77.

Meinesz, A., Astier, J.M., Bodoy, A., Cristiani., G. and Lefèvre, J.R., 1982. Impact de l'aménagement du domaine maritime sur l'étage infralittoral des Bouches du Rhône (France-Méditerranée Occidentale). Vie Milieu, 32(2): 115-124.

Meinesz, A., Boudouresque, C.F., Jeudy de Grissac, A., Lamare, J.-P., Lefèvre , J.-R. and Manche, A., 1985. Planning, management and protection of the Provence-Alpes-Cote d'Azur coastal areas: present state and prospects. Colloque Franco-Japonais d'Océanographie, Marseille, France, 16-21 Sept. 1985, 1: 133-142.

Miller, M.W., Aronson, R.B., and Murdoch, T.J.T., 2003. Monitoring coral reef macroalgae: different pictures from different methods. Bulletin of Marine Science, 72 (1): 199-206.

Munda, I.M., 1993. Changes and degradation of seaweed stands in the Northern Adriatic. Hydrobiologia, 260/261: 239-253.

Neto, A.I., 1997. Studies on Algal Communities de São Miguel, Azores, PhD thesis in Marine Biology, University de the Azores, Ponta Delgada, 1997.

Neto, A.I., 2000. Observations on the biology and ecology of selected macroalgae from the littoral of São Miguel (Azores). Botanica Marina 43: 483–498.

Pascoal, A., 2011. MAROV: Mapping of marine habitats in the Azores using robotic vehicles. Project homepage at ISR-IST, Lisbon:
http://welcome.isr.ist.utl.pt/project/index.asp?accao=showproject&id_project=7,
accessed 11/07/2011.

Pauly K. and De Clerck O., 2010. GIS-based environmental analysis, remote sensing and niche modeling of seaweed communities, In: Israel A., Einav R. and Seckbach J. (eds.), Seaweeds and their Role in Globally Changing Environments, Springer, 480 pp.
doi: 10.1007/978-90-481-8569-6.

Raffaelli, D., Bellb, E., Weithoffb, G., Matsumotoc, A., Cruz-Mottad, J.J., Kershawe, P., Parkere, R., Parryf, D. and Jones, M., 2003. The ups and downs of benthic ecology: considerations of scale, heterogeneity and surveillance for benthic–pelagic coupling. Journal of Experimental Marine Biology and Ecology, 285–286: 191–203.

Ready, J., Kaschner, K., South, A. B., Eastwood, P. D., Rees, T., Rius, J., Agbayani, E., Kullander, S. and Froese, R., 2010. Predicting the distributions of marine organisms at the global scale. Ecological Modelling, 221: 467-478.

Santos, R.S., Hawkins, S., Monteiro, L.R., Alves, M. and Isidro, E.J., 1995. Marine research, resources and conservation in the Azores. Aquatic Conservation: Marine and Freshwater Ecosystems, 5: 311–354.
doi: 10.1002/aqc.3270050406

Santos, R.S., Tempera, F., Cardigos, F. and Pascoal, A., 2001a. Mapping SACs for Management Purposes in the Azores. Habitat Mapping ICES Workshop "Mapping the Sea-floor", Bergen, Norway, February 2001.

Santos, R.S., Pascoal, A., Dando, P., Cardigos, F., Tempera, F., Oliveira, P., Silvestre, C. and Cardew, M., 2001b. Remote sensing at D. João de Castro bank: tools for biology and conservation studies. "5th Underwater Science Symposium", Southampton (United Kingdom).

Santos, R.S., 2011. MAROV: Mapping of Marine Habitats in the Azores Using Robotic Vehicles. project page at DOP, Horta: http://www.horta.uac.pt/projectos/marov/index1.html, accessed 11/07/2011.

Schaffelke, B., Smith, J.E. and Hewitt C. L., 2006. Introduced macroalgae - A growing concern. Journal of Applied Phycology, 18: 529-541.
doi: 10.1007/s10811-006-9074-2

Schmidt, O.C., 1929. Beiträge zur Kenntnis der Meeresalgen der Azoren. I. Hedwigia, 69: 95-113.

Schmidt, K., 1998. Fisheries biology: ecology's catch of the day. Science, 281 (5374): 192 - 193.
doi: 10.1126/science.281.5374.192

Seabra, M.I., Tempera, F. and Santos, R.S., 2005. Experiences in applying geographic information systems to marine conservation and ecology in the Azores. GeoInova, 11: 139-159.
http://www.horta.uac.pt/intradop/images/stories/perspages/ftempera/SIG_Seabra_Tempera_RSS_2005_a.pdf

Silva, LD., 2001. Plantas vasculares invasoras no Arquipélago dos Açores. PhD thesis. University of the Azores, Ponta Delgada. 200 pp.

Solan, M., Germano, J.D., Rhoads, D.C., Smith, C., Michaud, E., Parry, D., Wenzhöfer, F., Kennedy, R., Henriques, C., Battle, E.J.V., Carey, D.A., Iocco, L., Valente, R.M., Watson J. and Rosenberg, R., 2003. Towards a greater understanding of pattern, scale and process in marine benthic systems: a picture is worth a thousand worms. Journal of Experimental Marine Biology and Ecology, 285: 313–338.

Tempera, F., 2009. Benthic habitats of the extended Faial Island shelf and their relationship to geologic, oceanographic and infralittoral biologic features. PhD thesis, 23-06-09, University of St. Andrews, UK, 341 pp.
http://hdl.handle.net/10023/726

Tempera, F., Afonso, P., Morato, T., Prieto, R., Silva, M., Cruz, A., Gonçalves, J. and Santos, R.S., 2001. Biological assemblages of the Faial-Pico Channel SACs (in Portuguese). Departamento de Oceanografia e Pescas da Universidade dos Açores, Horta. Arquivos do DOP, Série Relatórios Internos, no. 7/2001, vi+95 pp.
http://www.horta.uac.pt/projectos/macmar/life/novidades/pdfs/SIC/Canal/Comunidades1.pdf

Tempera, F., Bates, R., and Santos, R.S., 2004. Mapping sublittoral benthic biotopes in Faial Island and neighbouring channel (Azores, Portugal). Geohab 2004 - Marine Geological and Biological Habitat Mapping, Galway (Ireland).

Tempera, F., and Santos, R.S., 2006. Marine habitat mapping activities in Portugal during 2005. Presented at the 2006 meeting of the "ICES Working Group on Marine Habitat Mapping", Galway (Ireland).

Thibaut, T., Manoni, P A, Mangialajo, L., Bottin, L. and Macic, V, 2009. Regression of the *Cystoseira* populations in the Mediterranean sea – the case of the french coast. ASLO Aquatic Sciences Meeting 2009, Nice, France, 25-30 January 2009.

Thomas, C. D., Cameron, A., Green, R. E., Bakkenes, M., Beaumont, L. J., Collingham, Y. C., Erasmus, B.. F. N., De Siqueira, M. F., Grainger, A., Hannah, L., Hughes, L., Huntley, B., Van Jaarsveld, A. S., Midgley, G. F., Miles, L., Ortega-Huerta, M. A., Peterson, A. T., Phillips, O. L. and Williams, S. E., 2004. Extinction risk from climate change. Nature, 427: 145-148.
doi: 10.1038/nature02121

Thrush, S.F. and, Dayton, P.K., 2002. Disturbance to marine benthic habitats by trawling and dredging: implications for marine biodiversity. Annual Review of Ecology and Systematics, 33: 449-473.
http://www.jstor.org/stable/3069270

Thuiller, W., Richardson, D.M., Pysek, P., Midgley, G.F., Hughes, G.O. and Rouget, M., 2005. Niche-based modelling as a tool for predicting the risk of alien plant invasions at a global scale. Global Change Biology, 11: 2234-2250.

Tyberghein, L., Verbruggen, H., Pauly, K., Troupin, C., Mineur, F. and De Clerck, O., 2011. Bio-ORACLE: a global environmental dataset for marine species distribution modelling. Global Ecology and Biogeography. Accepted, published on-line, 2011.
doi: 10.1111/j.1466-8238.2011.00656.x

Watling, L. and Norse, E.A., 1998. Disturbance of the seabed by mobile fishing gear: a comparison to forest clearcutting. Conservation Biology, 12 (6): 1180–1197.

Wiley, E.O., Mcnyset, K.M., Peterson, A.T., Robins, C.R. and Stewart, A.M., 2003. Niche modelling and geographic range predictions in the marine environment using a machine-learning algorithm. Oceanography, 16 (3): 120-127.

2 - The environment of Monte da Guia SAC and selected study sites

Table of contents

2.1 General surrounding hydrology

The location of Faial Island (Azores, Portugal, NE Atlantic) is illustrated in figure 1 within the general surrounding geostrophic surface transport showing the North Atlantic Current southern branch (NAC2) and Azores current (AzC) (Siedler and Onken, 1996, as in Tempera, 2009). The Azores Current, south of the Azores Archipelago, is part of the subtropical North Atlantic gyre and is found to originate in the area of the Southwest Newfoundland Rise (Klein and Siedler, 1989). In winter an almost uniform current connects this region of origin with the Azores Current, while a branching into two current bands is observed in summer, with the southern band forming a marked cyclonic loop.
Seasonal variations of the general patterns of the Azores currents are illustrated in Mariano (2011). Range of environmental conditions encountered in the open ocean water masses surrounding the Azores are described from remote sensing by several authors (Bashmachnikov *et al.*, 2004; Lafon *et al.*, 2004; Guimarães, 2008). High-resolution climatology of the north-Eastern Atlantic was also recently produced from Data Interpolating Variational Analysis (DIVA) of a recently compiled extensive data base of *in situ* measurements (Troupin *et al.*, 2010), providing up to date information on the average seasonal conditions around the archipelago.

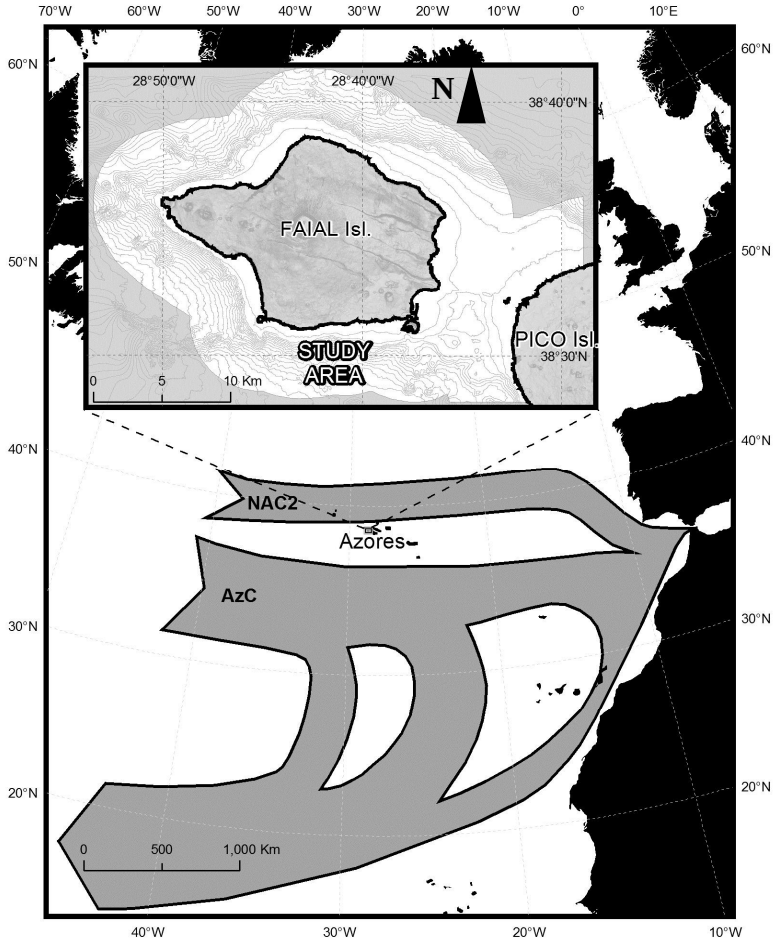


Figure 1 - Location of Faial Island within the general geostrophic surface transport showing North Atlantic Current southern branch (NAC2) and the Azores current (AzC) (Adapted by Tempera, 2009 from Siedler and Onken, 1996).

2.2 Physiography of the islands of the central group and Faial-Pico Channel

Among the islands of the central group of the Azorean islands, the three island of Faial, Pico and São-Jorge are close to each other. A high resolution map of their surrounding bathymetry (Tempera, 2009) shows the general abrupt slopes surrounding the islands, and the shallow character of the Faial-Pico channel contrastingly to the surroundings (Fig. 2). Acoustic mapping technics revealed the distribution of rocky seabed versus soft sediment substrates (Fig. 3; Tempera, 2009).

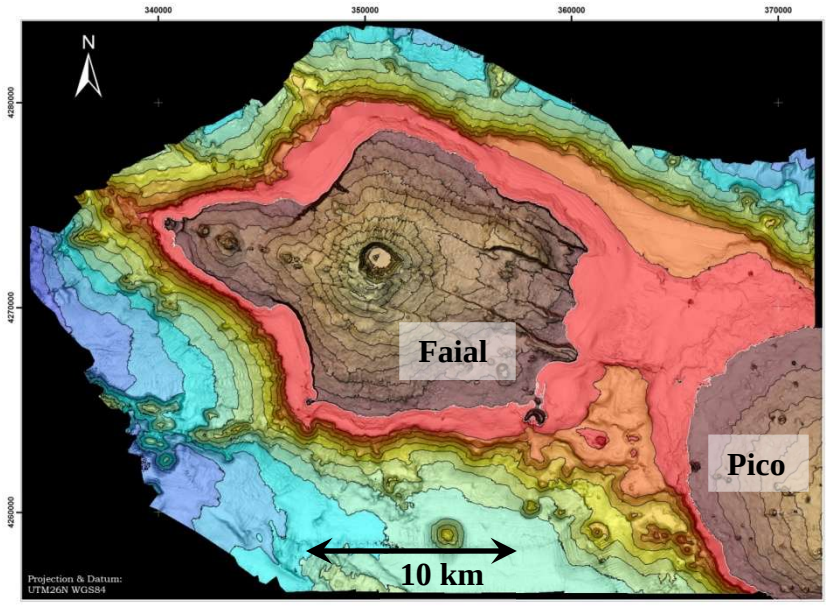


Figure 2 - High resolution color coded bathymetry around Faial Island showing the shallow Faial-Pico Channel; every contour line represents 100 m (Tempera, 2009).

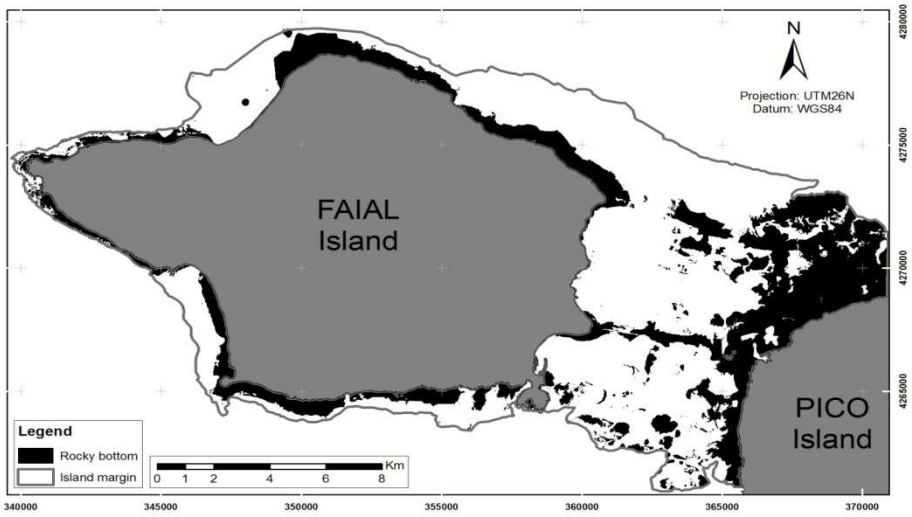


Figure 3 - Rocky seabed habitat identified within Faial shelf by acoustic methods, above the 100 m isobath (grey line) : black = rock; white = sand (Tempera, 2009).

2.3 The study sites within Monte da Guia SAC

In the SAC of Monte da Guia, several sites were explored (Fig. 4), to identify reference sites for the present investigation on population structures and dynamics of *Codium elisabethae.* Two study sites were selected.

The first site is located in the sheltered no-go reserve of the Caldeirinhas (point 1a in Fig. 4), constituted by 2 ancient volcano craters, now eroded and half opened to the sea. By 22 meters depth, the extensive rocky bottom of the outer crater is occupied by a dense *Codium elisabethae* population.

The second site is situated in a location experiencing more exposed conditions, at a distance of about 2 km from the first one. There, a more sparse population occupies rocky tables and boulders emerging at about 19 meters depth from shallow sandy deposits (site of Ponta Furada, point 2 in Fig. 4). The identification of this second site was carried out with scuba-diving explorations made possible by communication links allowing DOP divers transiting with scooters over wide areas to communicate with boat and record positions of several potential sites without surfacing in prevision of final selection dives.

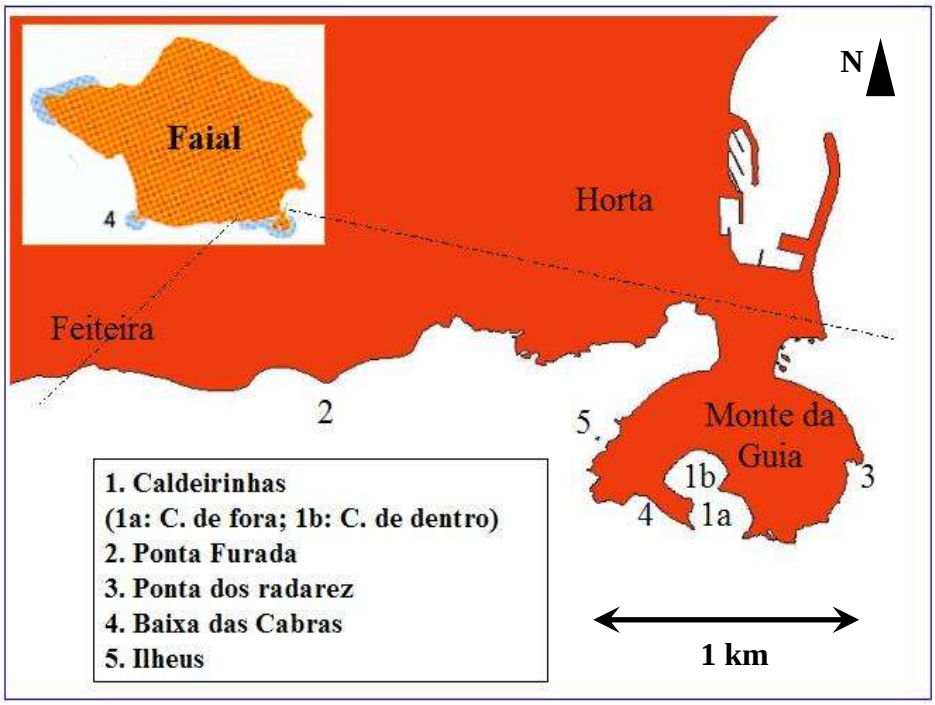


Figure 4 - Localization of the SAC of Monte da Guia (one of 3 Natura 2000 sites shaded in blue around Faial), with indication of particular sites occupied by *Codium elisabethae.*

These two selected sites are represented by black and yellow squares on a high resolution acoustic bathymetry map in figure 5.

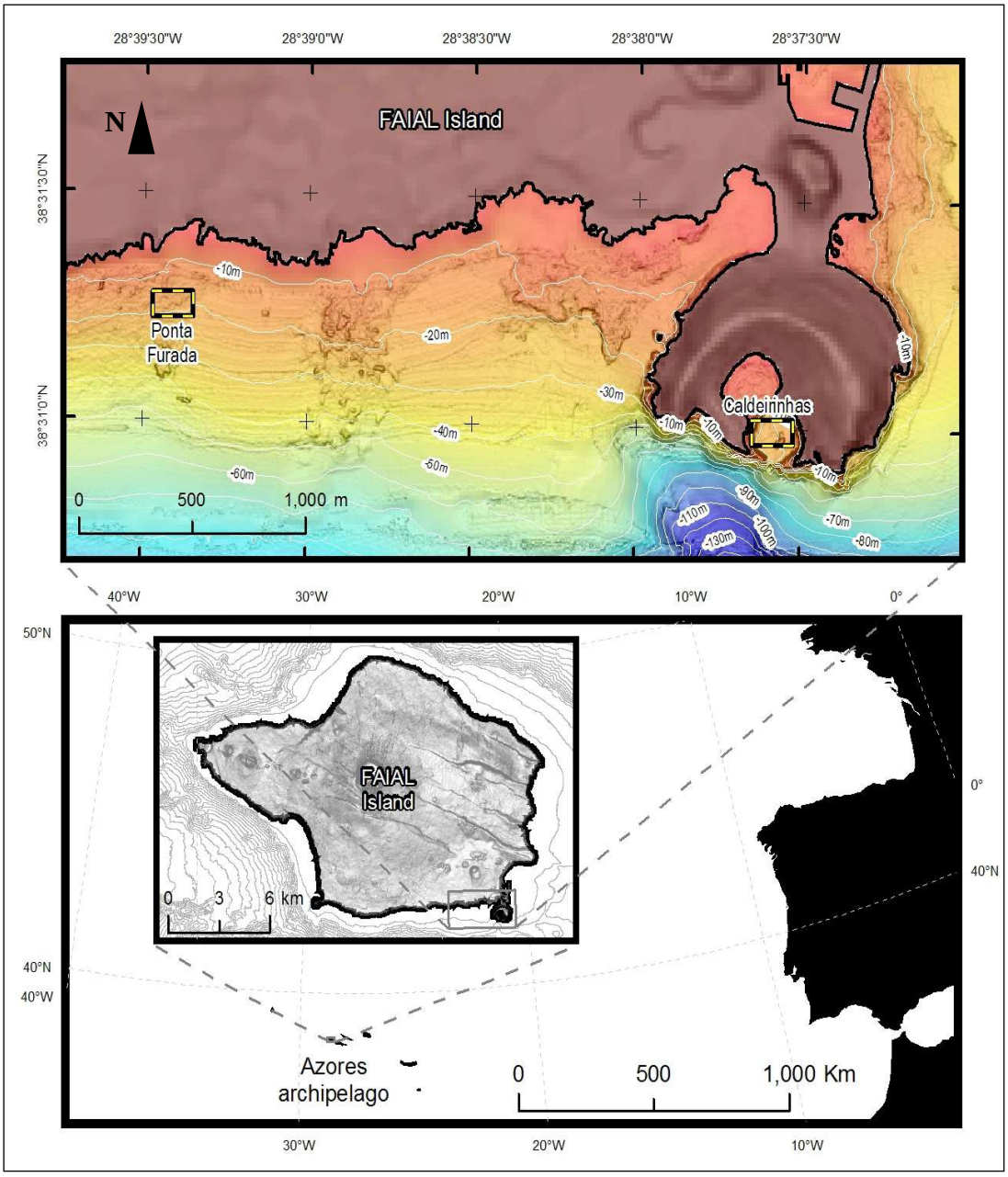


Figure 5 - Location and detailed bathymetry of the study sites Ponta Furada and Caldeirinhas within the "Monte da Guia" SAC (up) established at the southwestern Faial Island, Azores, North Atlantic (down).

2.4 The coastal hydrology

The general swell and wind statistics are summarized in figure 6. The seasonal thermal stratification of the water column is illustrated by average CTD profiles collected around the three islands of Faial, Pico and São-Jorge (Fig. 7).

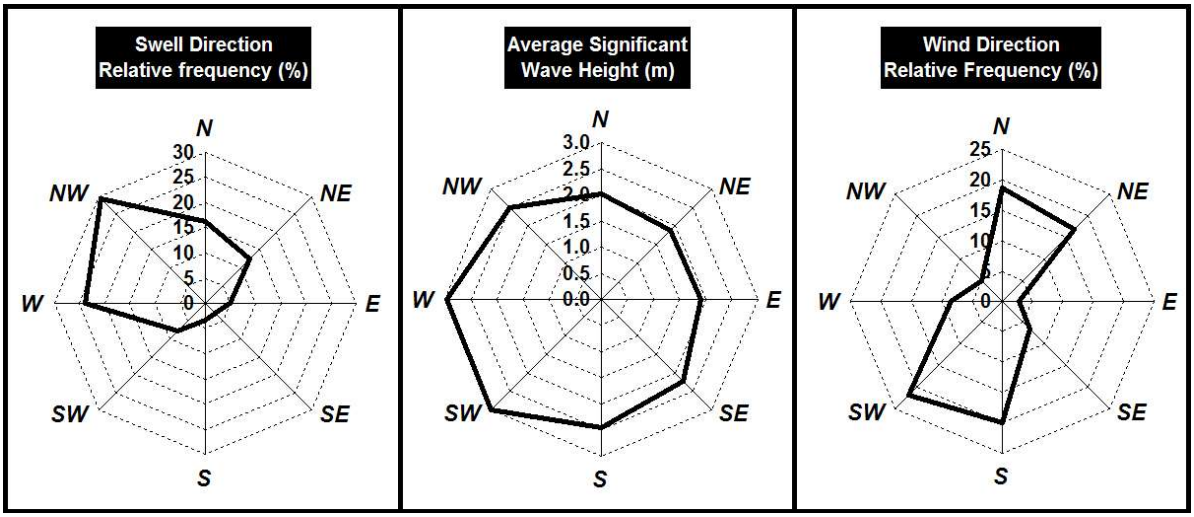


Figure 6 - Swell and wind statistics for Faial Island (adapted from Carvalho, 2003 and data from the Horta Meteorologic Observatory provided by Instituto de Meteorologia)

Middle Atlantic surface waters constitute an oligotrophic environment where scarce nutrient dynamics limit and condition the dynamic of primary production. Interaction between large scale hydrodynamics and geological structures can create localized upwellings bringing deeper nutrient rich waters to the photic zone and enhancing photosynthetic productivity, as known for seamounts but also most probably valid for particular shapes of islands, as identified from CTD data south and north of the Faial-Pico channel due to current and tidal effects (Bashmachnikov, personal communication; Bashmachnikov and Martins, 2006; Tempera, 2009) (Fig. 8). In addition to this, natural water surface runoff from islands slopes and groundwater discharges enrich coastal waters. Particularly preserved, the Azorean coastal zone nevertheless also receives some nutrients as consequence of developing anthropic presence and activities. For some cities, human population densities reach notably higher value during summer, corresponding to a period during which surface waters are more stratified and would thus, in total pristine conditions, be generally more depleted in nutrients. No extensive evaluation of nutrient in coastal seawater of Monte da Guia was found at the start of the study.

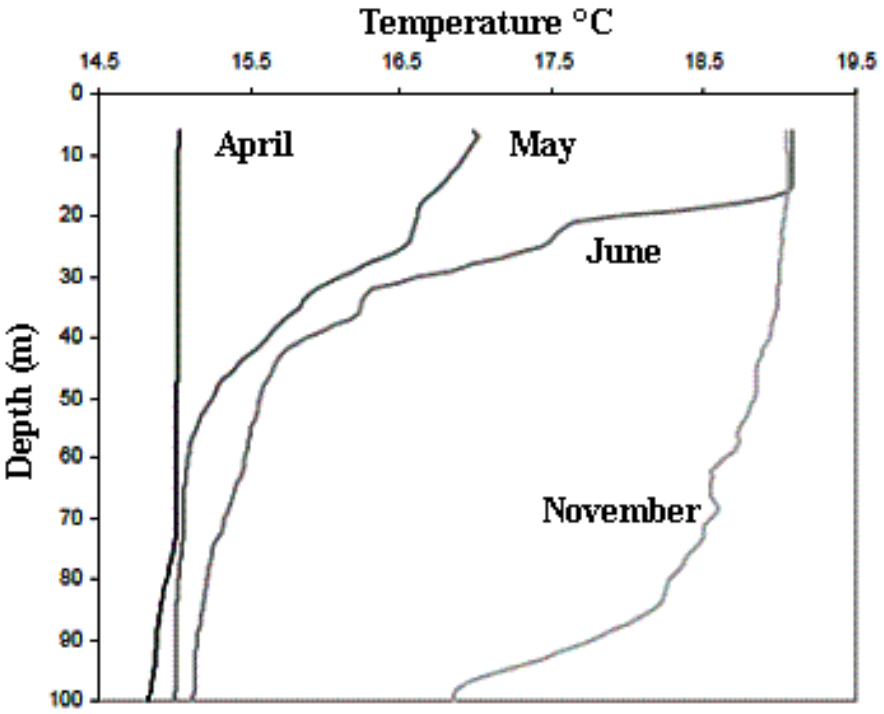


Figure 7 - Mean seasonal stratification of the water column around the Islands of Faial, Pico and São-Jorge (from CTD data courtesy of Bashmachnikov)

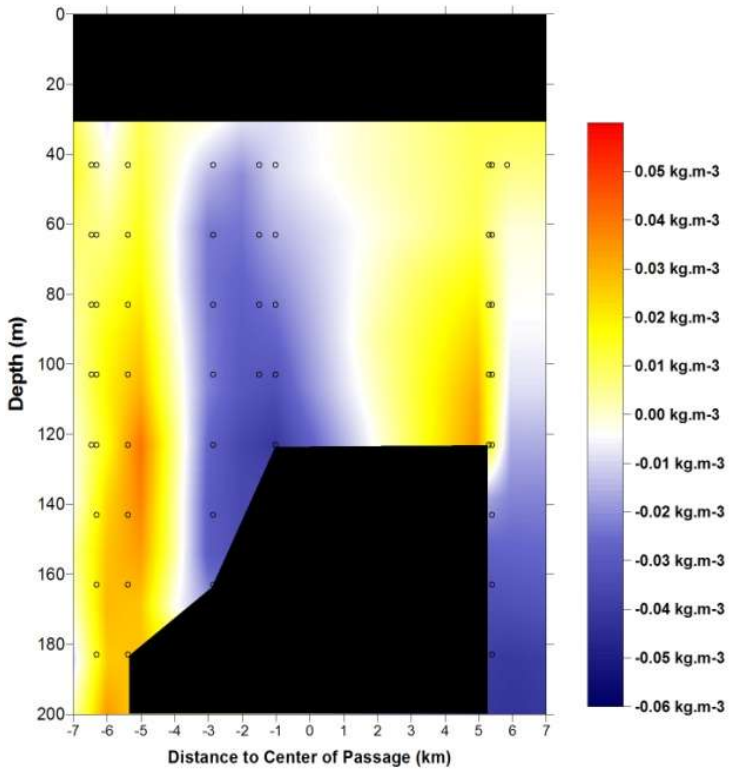


Figure 8 - Density anomalies (kg.m-3) from a South (left) to North (right) cross-section through Faial-Pico Channel. Blue shades are lower than average densities whilst warm colors are higher than average densities. White is for values around the average density. Points represent measurements along CTD casts used in the interpolation (Tempera, 2009).

At the start of the present study, only few studies were available concerning the coastal hydrology and at the close scale of Monte da Guia SAC benthic habitats (Carvalho, 2003; Duarte, 1997). Generally, extensive in *situ* data or modelling tools were missing to describe precisely the spatio-temporal variability of environmental conditions encountered at bottom in general and for our study sites in particular. Some seabed temperature time series were recorded in few locations around Monte da Guia as support to reproduction and telemetry studies on territorial fishes (Morato *et al.*, 2003), but not at close proximity to our reference sites neither during the whole planned monitoring period. Few CTD profiles happened to be collected south of the Monte da Guia SAC but excessively offshore to reveal any variability pattern of the water column structure within the SAC itself. A modelling study described the effects of passing tides on sea level variations and currents at the proximity of the Faial-Pico channel (Duarte, 1997), but does not integrate other important forcings (currents at boundary conditions, surface winds, atmospheric pressure) and its spatial resolution did not fit the needs of our close scale interests. This situation drove the need to implement a detailed monitoring of benthic habitat conditions for the study sites selected for the present investigation.

More recently, wave and current models were implemented around Faial and allowed to evaluate the general of exposure of benthic habitats for spatial distribution modelling of seaweeds (Tempera, 2009).

2.5 The biotic environment

European efforts aiming at nature conservation provided resources to develop various projects related to the management of macaronesian sites included in the Natura 2000 network, with extended presentations on living resources and recent concerning on-going investigations accessible to the public (DOP_MACMAR, 2011).

In the context of the MAROV project, numerous marine habitat and biodiversity mapping applications have been undertaken for various Marine Special Areas of Conservation (SACs) all around the Azores (Cardigos and Tempera, 2002; Santos *et al.*, 2001a and 2001b; Seabra *et al.*, 2005; Tempera *et al.*, 2004; Tempera and Santos, 2005 and 2006).

The channel between the islands of Faial and Pico contains a series of designated areas, as Monte da Guia, Baixa do Sul and Madalena islets (Cardigos and Tempera, 2002). These areas were used as primary sites for various research projects, where the scientific techniques were put together, tested and, eventually, implemented. The work developed included visual estimation of abundance and diversity of benthic and fish assemblages by SCUBA diving, ROV and drop down cameras. These efforts have resulted in the definition of biotopes and mapping of their distribution (Tempera *et al.*, 2001a). Together with an analysis of the socio-economical context of the SACs, this research formed the basis for preliminary management plans that allowed public hearing (Tempera *et al.*, 2001b).

Pelagic and benthic biological communities present in the Faial-Pico channel and of the Monte da Guia SAC were described in Tempera *et al.* (2001a), with specific attention to benthic macroalgae, reef fishes, and cetaceans.

2.6 The seaweed communities

The phytogeography of seaweeds of the Azores was reviewed by Prud'Homme Van Reine (1988). New records of benthic macroalgae are still occurring in the Azores (Parente and Neto, 2000; Tittley, 2002) as 186 species were listed in South and Tittley (1986) for this archipelago and a decade of study has increased the total to 256 species in Neto (1994) then 360 species (Parente and Neto, 2000; Tittley *et al.* 1998 and 2001). Finally on-going work with this group of flora led to the current number of approximately 400 species identified (Tittley *et al.*, 2009). Schmidt (1931, in Neto 1994) listed 8 species as endemic for the Azores, but most of these were never confirmed in posterior work (Netbiome, 2011). According to Tittley (2002), even at better-known locations than the Azores, nomenclatural and taxonomic information continues to change and new algal records continue to be accumulated. Wallenstein *et al.* (2008a and 2008b) exploited a method based on point count sampling and identified ten algal biotopes in the Islands of São-Miguel and Santa-Maria (Azores). They exploited depth as sole determining factor for communities and produced vertical zonation for both islands.

Some of the new records in this remote middle Atlantic archipelago do show some local invasive "behavior", as *Caulerpa webbiana* present in the SAC of Monte da Guia (Amat *et al.*, 2008).

Within the SAC of Monte da Guia, Tempera *et al.* (2001a) listed a total of 47 different macroalgae (Table 1). Frequent algae encountered are *Corallinaceae* spp., *Codium elisabethae*, *Dictyota* spp., *Halopteris filicina, Padina pavonica, Zonaria tournefortii.*

Table 1 - List of different macroalgae observed in the SAC of Monte da Guia (Tempera *et al.*, 2001a)

Rhodophyta	**Phaeophyta**	**Chlorophyta**
25 species	14 species	8 species
Amphiroa sp.	***Cladostephus spongiosus***	***Anadyomenaceae spp.***
Asparagopsis armata	***Colpomenia spp.***	***Microdictyon calodictyon***
Asparagopsis taxiformis	***cf. Cutleria multifida (fase Aglaozonia)***	***Bryopsis sp.***
Ceramiales indet. sp.1	***Cystoseira cf. abies-marina***	***Cladophora sp. 1***
Ceramiales indet. sp.2	***Dictyota cf. adnata***	***Cladophora sp. 2***
Ceramiales indet. sp.3	***Dictyota dichotoma***	***Codium elisabethae***
Ceramiales indet. spp.	***Dictyota cf. linearis***	***Codium spp.***
Ceramiales polissifonadas indet.	***Halopteris filicina***	***Valonia utricularis***
Corallina sp.	***Nemoderma tingitana***	
Corallinacea indet.	***Padina pavonica***	
Delesseriacea spp.	***cf. Phaeophyta filamentosas indet.***	
Gelatinosa cromada	***Stypocaulon scoparium***	
Gelatinosas indet.	***Stypopodium zonale***	
Gelidium pusillum	***Zonaria tournefortii***	
Haliptylon/Jania spp.		
Hypnea spp.		
Peyssonnelia sp.1		
Peyssonnelia sp.2		
Peyssonnelia sp.4		
Plocamium cartilagineum		
Pterocladia capillacea		
Rhodophyta indet.		
Rhodophyta indet.		
Rhodymenia holmesii		
Symphyocladia marchantioides		

2.7 The importance and distribution of *Codium elisabethae* populations

Among these algae, the Chlorophyta *Codium elisabethae* can be considered an important species within the seaweed community of Monte da Guia SAC but also in the Azores in general. As a matter of fact, it is widely distributed in Monte da Guia and all around the islands (Tempera *et al.*, 2001a and 2009) (Figs. 9, 10 and 11), and it is adaptated to extreme conditions habitats as important depths or proximity to hydrothermal vent fluids (Cardigos *et al.*, 2005). *Codium elisabethae* vertical distribution ranges from few meters depth till 40 meters, and reaches a maximum density around 20-25 meters, based on averaged SACFOR abundance (Connor and Hiscock, 1996) as evaluated by Tempera (2009) (Fig. 12). Further, its local densities and cover rate range in many places from important to dominant (SACFOR scales; Tempera *et al.*, 2001a), making from it the main vegetal biomass in several areas.

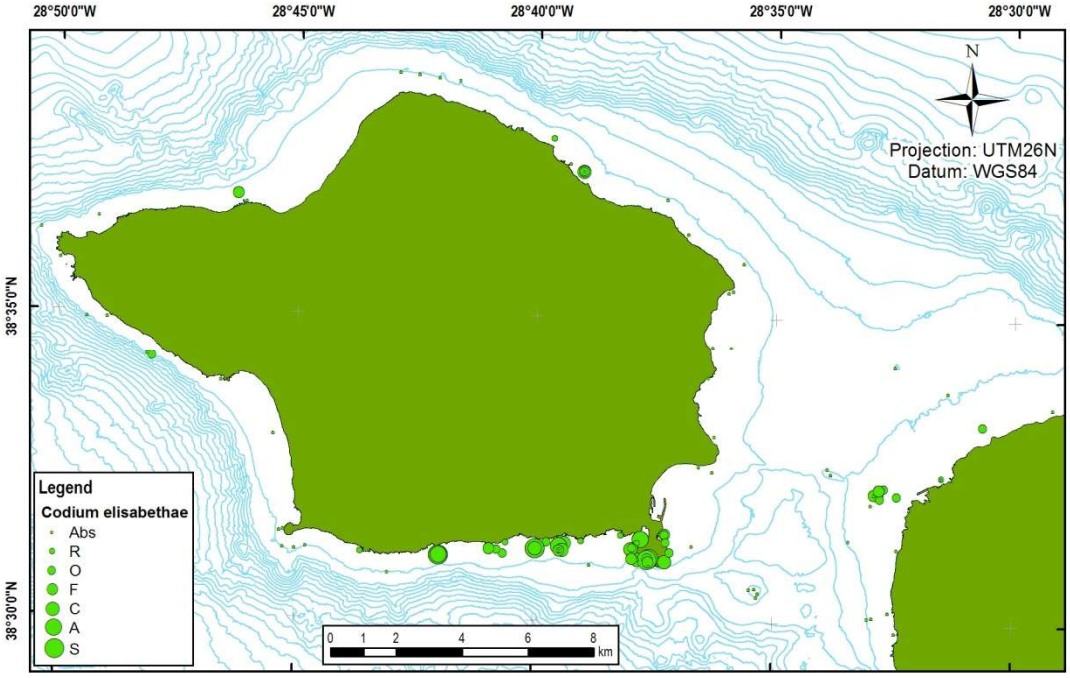


Figure 9 - Spatial distribution of *Codium elisabethae* observations and respective SACFOR qualitative abundance score (abundant to superabundant in Ponta Furada and Caldeirinhas); (Tempera, 2009)

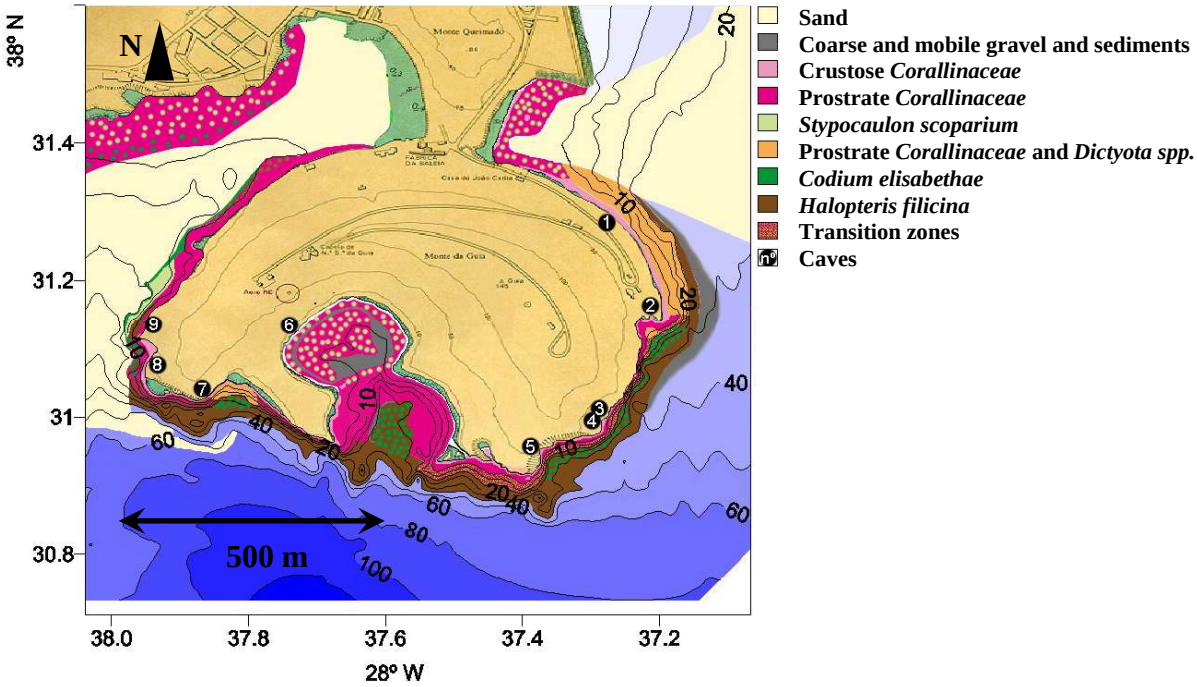


Figure 10 - Approximate distribution of algal populations around the volcanic cone of Monte da Guia. Composition based on prospections carried out during summer 1999. Blue shades represent only the bathymetry (Tempera *et al.*, 2001a);

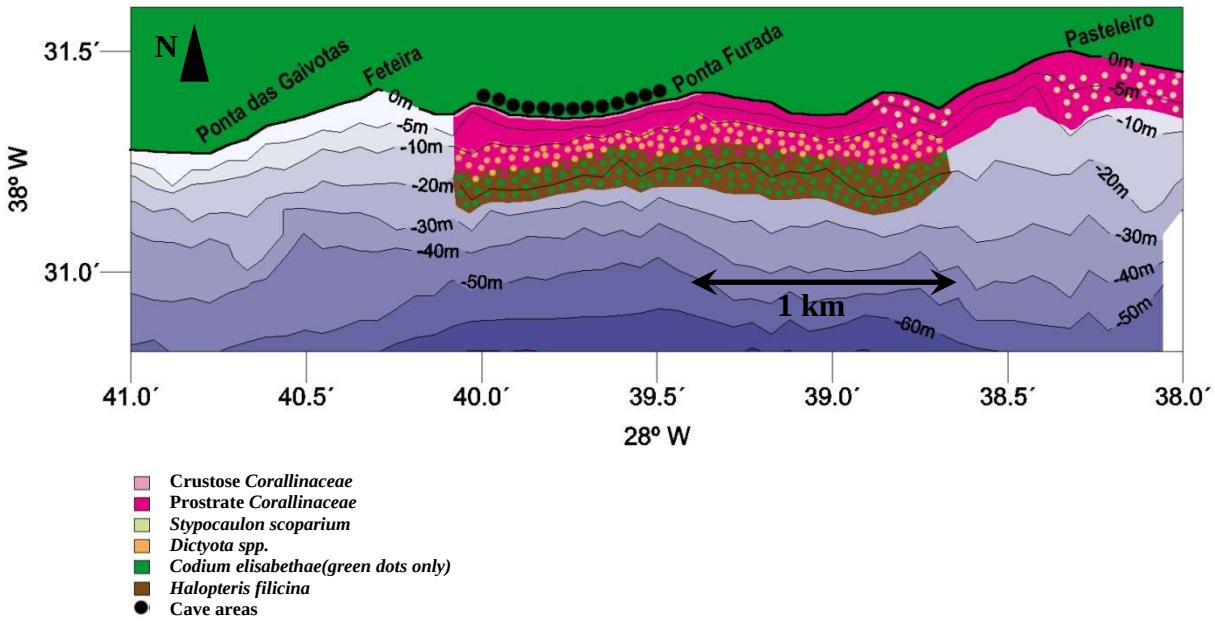


Figure 11 - Approximate distribution of algal populations on rocky seabed in a transect of the south-east coast of Faial island (Pasteleiro-Feteira). Composition based on prospections carried out during summer 1999. Blue shades represent only the bathymetry (Tempera *et al.*, 2001a).

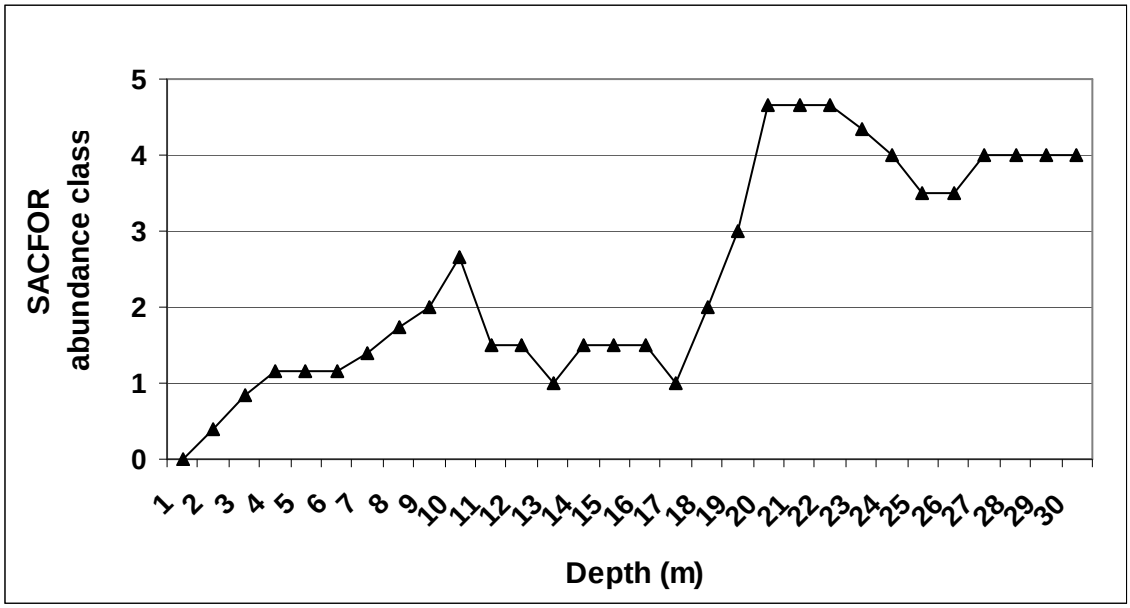


Figure 12 - Depth distribution of averaged SACFOR abundance class of *Codium elisabethae* around the coasts of Faial and Pico islands (Tempera, personal communication).

Around the Faial in the Pico-Faial channel, Tempera (2009) undertook a very interesting predictive study of the spatial distribution of numerous seaweeds (articulated *Corallinaceae*, *Codium elisabethae*, *Dictyota* spp., *Halopteris filicina*, *Padina pavonica* and *Zonaria tournefortii*) versus environmental parameters (depth, slope, swell exposure, maximum tidal currents, SST and chl-a concentration). As a result, a refined understanding concerning the ecological habitat fitting of these species was produced, and the degree of consistency between the prediction provided by these models and the observed distributions will allow new hypothesis for future ecological studies. Concerning *Codium elisabethae*, the model reproduced algal presence only in places where the populations are very abundant (Monte da Guia; Fig. 13), with relatively consistent general spatial patterns there (as compared to *in situ* visual estimates; Figs. 9 and 10), but with low probability of predicted SACFOR class resulting of clear abundance underestimation (Fig. 14).

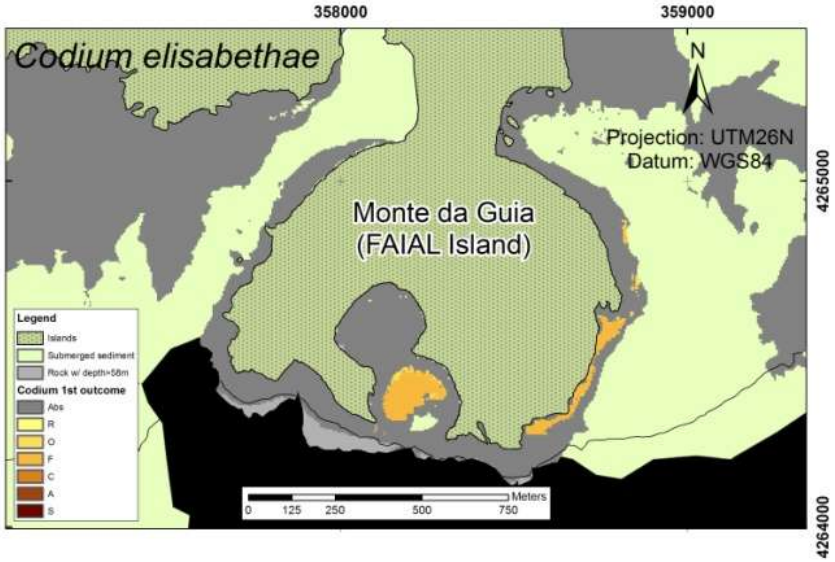


Figure 13 - *Codium elisabethae*: abundance class prediction (most probable outcome) based on spatialized consideration of the significant explanatory variables depth, slope, exposure to swell and exposure to tidal currents); (dark grey = Absent; yellow = Rare; brown = Super-abundant; Tempera, 2009).

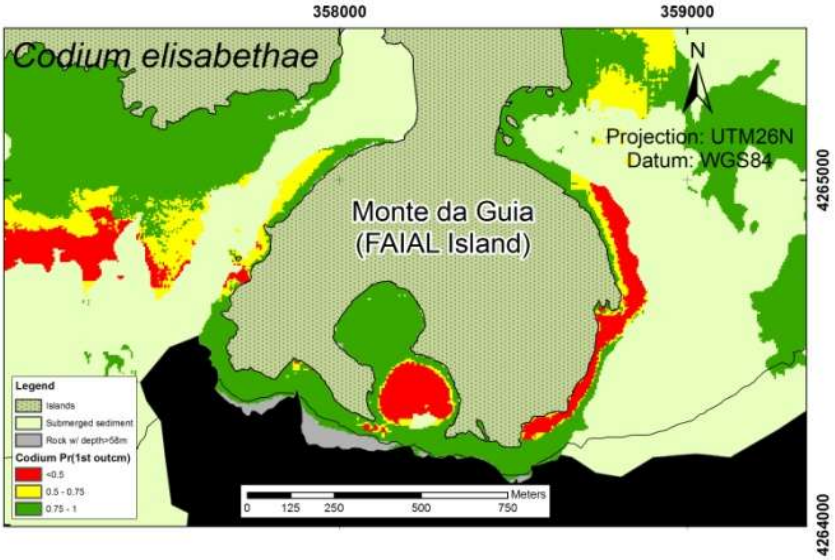


Figure 14 - *Codium elisabethae*: probability of the predicted abundance class (red = below 50%; yellow= 50 to 75 %, green= 75 to 100 %) (Tempera, 2009).

Scuba diving explorations at the maximum density depth range of *C. elisabethae* revealed contrasting populations densities and structures according to local environment, as of the two selected study sites (Fig. 15). The result of distribution modelling studies suggests additional factors are required to predict population densities. This raises numerous interesting questions about the auto-ecology of these algae. What elements of the habitat constraints but also of the life cycle dynamics do structure the local populations? How stable in time are the characteristics of those local populations? What is the range of their primary production contribution to the benthic trophic chains? These first questions are addressed by the present research, and they open the way to longer term monitoring interests. Can some changes in the population static or dynamic parameters of these long living benthic organisms be exploited as integrating indicators of environmental changes? Can some of these populations be considered robust and stable and others just as sensitive transitory colonizers?

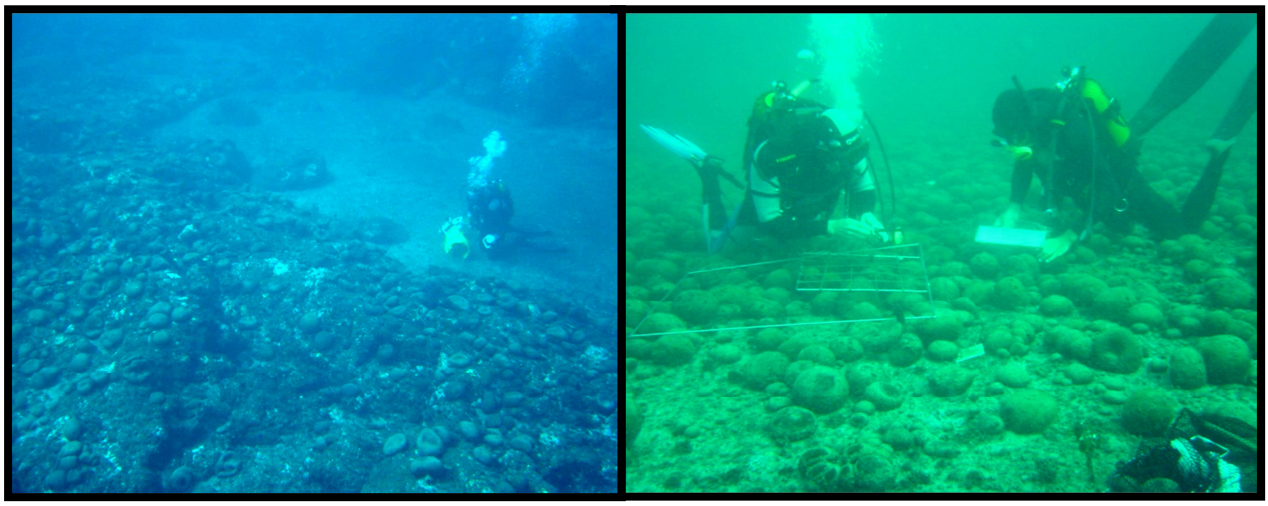


Figure 15 - *Codium elisabethae* stands within the sites of Ponta Furada (left) and Monte da Guia (right).

2.8 References

Amat, J.N., Cardigos, F. and Santos, R.S., 2008. The recent northern introduction of the seaweed Caulerpa webbiana (Caulerpales, Chlorophyta) in Faial, Azores Islands (North-Eastern Atlantic). Aquatic Invasions, 3(4): 359-360.
doi: 10.3391/ai.2008.3.4.xx

Bashmachnikov, I., Lafon, V. and Martins, A., 2004. Sea surface temperature distribution in the Azores region. Part II: space-time variability and underlying mechanisms. Arquipélago. Life and Marine Sciences, 21A: 19-32.

Bashmachnikov, I. and Martins, A., 2006. Upper layer water structure and dynamics in the region of Triangle (Faial, Pico, S.Jorge) from the CTD and moorings data, obtained during ORPAM cruises. Internal report, Oceanography section, DOP. May 2006, 20 pp.

Cardigos, F. and Tempera, F., 2002. Mapping of marine biodiversity at marine Special Areas of Conservation (SACs) in the Azores. Implementation of large-scale long-term MARine BIOdiversity research in Europe. Newsletter 3.
http://www.biomareweb.org/3.5.html

Cardigos, F., Colaço, A., Dando, P.R., Ávila, S.P., Sarradin, P.-M., Tempera, F., Conceição, P., Pascoal, A. and Santos, R.S., 2005. Shallow water hydrothermal vent field fluids and communities of the D. João de Castro Seamount (Azores). Chemical Geology, 224: 153-168.

Carvalho, F., 2003. Elementos do clima de agitação marítima no Grupo Central dos Açores (Mar Alto). Instituto de Meteorologia, Portugal. 98 pp.

Connor, D. and Hiscock, K., 1996. Data Collection Methods. In Hiscock, K. (ed.). Marine Nature Conservation Review: Rationale and Methods. Joint Nature Conservation Committee, Coasts and seas of the United Kingdom, MNCR series, Peterborough: 51-65, 126-158.

DOP_MACMAR, 2011. EU - LIFE- projects in the macaronesian region;
http://www.horta.uac.pt/projectos/macmar/ogamp/index.html, accessed 15/06/0211.

Duarte, R.M.M.D., 1997. Simulação numérica de correntes induzidas pelas marés: o caso Faial-Pico no arquipélago dos Açores. Licenciatura thesis. Departamento de física, Faculdade de Ciências da Universidade de Lisboa, Lisboa. 90 pp.

Guimarães, J.V.S.M., 2008. Sea Surface Temperature (SST) and Ocean color (OC) pattern relationships on an annual and seasonal basis in the Subtropical NE Atlantic, using satellite (AVHRR and MODIS) data (2002-2006). Relatorio de estagio, Licenciatura em biologia marinha do Departamento de biologia; Departamento de Oceanografia e Pescas, Universidade dos Açores, Horta. 64 pp.

Klein, B., and G. Siedler, 1989. On the origin of the Azores current. Journal of Geophysical Research, 94(C5): 6159–6168.
doi:10.1029/JC094iC05p06159.

Lafon, V., Martins, A., Figueiredo, M., Melo Rodrigues, M.A., Bashmachnikov, I., Mendonça, A., Macedo, L. and Goulart, N., 2004. Sea surface temperature distribution in the Azores region. Part: I: AVHRR imagery and *in situ* data processing. Arquipélago. Life and Marine Sciences, 21A: 1-18.

Mariano, A.J., 2011. http://oceancurrents.rsmas.miami.edu/atlantic/azores_3.html, accessed 15/08/11.

Morato, T., Afonso, P., Lourinho, P., Nash, R.D.M. and Santos, R.S., 2003. Reproductive biology and recruitment of the white sea bream in the Azores, Journal of Fish Biology, 63(1): 59-72. doi: 10.1046/j.1095-8649.2003.00129.x

Netbiome project, 2011. http://netbiome.org/index.php?option=com_content&view=article&id=65&catid=51&showall=1, accessed 15/06/2011.

Neto, A.I., 1994. Checklist of the benthic marine algae of the Azores. Arquipélago Life and Marine Sciences 12A: 15-24.

Parente, M.I. and Neto, A.I., 2000. New records of benthic marine red algae (Ceramiales: Rhodophyta) from the Azores. Arquipélago. Life and Marine Sciences Sup. 2 (A): 53-61.

Prud'Homme Van Reine, W.F., 1988. Phytogeography of seaweeds of the Azores. Helgoländer Meeresuntersuchungen 42: 165–185.

Santos, R.S., Tempera, F., Cardigos, F. and Pascoal, A., 2001a. Mapping SACs for management purposes in the Azores. Habitat mapping ICES workshop "Mapping the Sea-floor", Bergen, Norway, February 2001.

Santos, R.S., Pascoal, A., Dando, P., Cardigos, F., Tempera, F., Oliveira, P., Silvestre, C. and Cardew, M., 2001b. Remote sensing at D. João de Castro bank: tools for biology and conservation studies. “5th Underwater Science Symposium”, Southampton (United Kingdom).

Schmidt, O.C., 1931. Die marine vegetation der Azoren in ihren grundzügen dargestellt. Bibliotheca Botanica 25(102): 1-116.

Seabra, M.I., Tempera, F. and Santos, R.S., 2005. Experiences in applying geographic information systems to marine conservation and ecology in the Azores. Geoinova, 11: 139-159. http://www.horta.uac.pt/intradop/images/stories/perspages/ftempera/SIG_Seabra_Tempera_RSS_2005_a.pdf

Siedler, G. and Onken, R., 1996. Eastern recirculation. In: Krauss, W. (ed.). The warm water sphere of the North Atlantic ocean. Gebrüder Borntraeger, Berlin. pp. 339-364.

South, G.R. and Tittley, I., 1986. A checklist and distributional index to the benthic marine algae of the North Atlantic Ocean. Huntsman Marine Laboratory and British Museum (Natural History), St. Andrews and London. pp. 76.

Tempera, F., 2009. Benthic habitats of the extended Faial Island shelf and their relationship to geologic, oceanographic and infralittoral biologic features. PhD thesis, 23-06-09, University of St. Andrews, UK, 341 pp. http://hdl.handle.net/10023/726

Tempera, F., Afonso, P., Morato, T., Prieto, R., Silva, M., Cruz, A., Gonçalves, J. and Santos, R. S., 2001a. Biological assemblages of the Faial-Pico channel SACs (in Portuguese). Departamento de Oceanografia e Pescas da Universidade dos Açores, Horta. Arquivos do DOP, Série Relatórios Internos, nº 7/2001, vi+95 pp. http://www.horta.uac.pt/projectos/macmar/life/novidades/pdfs/SIC/Canal/Comunidades1.pdf

Tempera, F., Afonso, P., Morato, T., Gubbay, S., Dentinho, T., Silva, M., Prieto, R., Cardigos, F., Pitta, M.J., and Santos, R.S., 2001b. Technical-scientific management proposal for the Faial-Pico Channel SACs (in Portuguese). Departamento de Oceanografia e Pescas da Universidade dos Açores, Horta. Arquivos do DOP. Série Relatórios Internos, nº 8/2001, viii+76 pp.

Tempera, F., Bates, R. and Santos, R.S., 2004. Mapping sublittoral benthic biotopes in Faial Island and neighbouring channel (Azores, Portugal). Geohab 2004 - Marine Geological and Biological Habitat Mapping", Galway (Ireland).

Tempera, F. and Santos, R.S., 2005. Projects and tasks related to habitat mapping. Recent and ongoing work in the Azores (Portugal). 2005 meeting of the ICES Working Group on Marine Habitat Mapping (WGMHM), Bremerhaven (Germany).

Tempera, F. and Santos, R.S., 2006. Marine habitat mapping activities in Portugal during 2005. 2006 meeting of the ICES Working Group on Marine Habitat Mapping, Galway (Ireland).

Tittley, I., Neto, A.I. and Farnham, W.F., 1998. Marine algae of the island of Flores, Azores: ecology and floristics. Boletim do Museu Municipal do Funchal. Suplemento 5: 463-479.

Tittley, I., Neto, A.I. and Farnham, W.F. and Parente, M.I., 2001. Additions to the marine algal (seaweed) flora of the Azores. Botanica Marina 44: 215-220.

Tittley, I., 2002. Seaweed diversity in the North Atlantic Ocean. Arquipélago. Life and Marine Sciences, 19A: 13-25.

Tittley, I., Neto, A.I. and Parente, M.I., 2009. The Marine algal (seaweed) flora of the Azores: additions and amendments 3. Botanica Marina, 5: 7-14.

Troupin, C., Machin, F., Ouberdous, M., Sirjacobs, D., Barth, A. and Beckers, J. M., 2010. High-resolution climatology of the northeast Atlantic using Data-Interpolating Variational Analysis (Diva). Journal of Geophysical Research-Oceans, 115 (C08005), 20 pp.

Wallenstein, F.M., Neto, A., Álvaro, N.V. and Santos, C.I., 2008a. Algae-based biotopes of the Azores (Portugal): spatial and seasonal variation. Aquatic Ecology, 42(4): 547-559. doi: 10.1007/s10452-007-9134-y

Wallenstein, F.M., Neto, A., Álvaro, N.V. and Tittley, I., 2008b. Subtidal rocky shore communities of the Azores: developing a biotope survey method. Journal of Coastal Research, 1: 244 – 249. doi: 10.2112/05-0590.1

3 - The macroalga *Codium elisabethae*

Table of contents

3.1 Systematics of the *Codium elisabethae*

The *Codium* genus classification evolved through time as described by Meinesz (1980). According to the latest contribution made by Silva in 2005 on the Algaebase.org web site and confirmed by Guiry and Guiry (2011), the classification of the macroalga *Codium elisabethae* is today considered as part of the following taxonomic groups:

Empire	*Eukaryota*
Kingdom	*Plantae*
Phylum	*Chlorophyta*
Class	*Ulvophyceae*
Order	*Bryopsidales*
Family	*Codiaceae*
Genus	*Codium*

This traditional taxonomy is presented with morphological considerations (below the Kingdom level) in section 3.1.1 to 3.1.5. Section 3.1.6 describes the species *Codium elisabethae*, with comments on the similar species *Codium bursa*.

3.1.1 The Phylum: *Chlorophyta*

According to Pascher (1914), as to Adl *et al.* (2005), the *Chlorophyta* Phylum characteristics can be synthesized by the presence of flagella of swimming cells in pairs or multiples of two; a stellate structure linking nine pairs of microtubules at basal body transition zone; single or stacked thylakoïds; plastid with two membranes without periplastid endoplasmic reticulum; starch presence inside plastid; glycolate dehydrogenase present; a cell wall of cellulose when present. Cell division with phragmoplast is generally not found in the *Chlorophyta* (López-Bautista *et al.*, 2003). Some of the characters listed above are obviously not unique to the Chlorophyta, such as the presence of flagella or cellulosic cell wall.

3.1.2 The Class: *Ulvophyceae*

The *Codium* genus was long considered belonging to a specific class called *Bryopsidophyceae,* as described in Bessey (1907), as to Silva (1980). Current classification recognizes the order Bryopsidales within the class *Ulvophyceae*, as described in Leliaert *et al.* (2011a).

3.1.3 The Order: *Bryopsidales*

The Order of the *Bryopsidales* (Schaffner, 1922, as in Brodie *et al.*, 2007) refers to the *Bryopsidaceae* family and counts 10 Families amongst which many well-known families widelely represented in the Mediterranean (Guiry and Guiry, 2011) as the *Codiaceae* (*Codium bursa, Codium fragile, Codium corraloides*), *Halimedaceae* (*Halimeda tuna*), *Caulerpaceae* (*Caulerpa racemosa, Caulerpa taxifolia, Caulerpa prolifera*), *Udoteaceae* (*Flabellia petiolata*). One less known Familly is the *Pseudocodiaceae* (Hillis-Colinvaux, 1984), holding only one genus called *Pseudocodium* (Weber-van Bosse, 1896), macroscopically resembling *Codium* spp. The genus is also similar to *Codium* in containing mannan in siphon walls.

Microscopically, *Pseudocodium* appears more similar to *Halimeda*, since it consists of a core of medullary siphons surrounded by a cortex of swollen, cohering utricles (medullary branches) which are hexagonal or polygonal in surface view. It also lacks septa associated with reproductive structures. In a variety of morphological aspects *Pseudocodium* seems intermediate between *Codium* and *Halimeda*, leading to the family name *Pseudocodiaceae* being proposed.

3.1.4 The Family: *Codiaceae*

The *Codiaceae* familly (Kützing, 1843), as referred by Silva (1980), was earlier classified in the order of the *Siphonales* (Blackman and Transley, 1902), uniting all coenocytic *Chlorophyceae*. This order has been reorganized progressively following progresses of the systematics in this field. Feldmann (1954) proposed an important clarification based on the type of plasts presents (chloroplasts only or amyloplasts also) and on the type of reproductive cycle and the characteristics of the gamets. The history of evolution of this classification and its state in 1980 are described by Meinesz (1980). The *Codiaceae* family is now classified (Brodie *et al.*, 2007; algae base) within the order called *Bryopsidales* (Schaffner, 1922), as described above.

3.1.5 The Genus: *Codium*

The number of species known to the *Codium* (Stackhouse, 1797) genus was estimated to 120 by Chacana *et al.* (2003). There are today 135 species names flagged as currently accepted taxonomically in the AlgaeBase at present (Guiry and Guiry, 2011). Several others are either considered as synonyms, as being of uncertain taxonomic status subjected to some verification nomenclaturally.

The thallus is described (Guiry and Guiry, 2011) as spongy, anchored to rocks or shells by a weft of rhizoids and varying in size from 1 cm to 10 m long. Habitually varying in shape from widely applanate, pulvinate, digitaliform, globular, petaloid, membraniform, or dichotomously branched; erect or repent; branches are wholly terete or variously flattened, at times anastomosing. The morphological diversity of *Codium* genus was illustrated by Verbruggen *et al.*, (2007) (Fig. 1A-E). The internal structure is composed of a colorless medulla of densely intertwined siphons and a green palisade-like layer of vesicles (Fig. 1F) called utricles of various shapes (Fig. 1G-I) and showing variable sizes across species, ranging from 40-550 µm width and 400-6500 µm length (Brodie *et al.*, 2007). The Organelles, including innumerable nuclei and discoid chloroplasts (but no amyloplasts) are confined to a layer of cytoplasm appressed to a wall of which mannan is an important constituent. The chloroplasts lack pyrenoids. Carotenoid pigments are present and include siphaxanthin and siphonein. The septa (plugs), formed by centripetal deposition of wall material, are incomplete. The utricles arise primarily by enlargement of sympodial branches of medullary siphons, secondarily by budding or by production of additional utricle-forming medullary siphons from basal portion of existing utricles. Mature utricles are cylindrical or clavate with an apical wall usually thickened and often ornamented in a pattern characteristic of particular species. Rhizoidal siphons, which become buried in the medulla, are also produced from basal portion of utricles. Colorless hairs, each with a basal plug, are produced by utricles shortly below their apices and are caducous at the plug, which remains as a prominent scar.

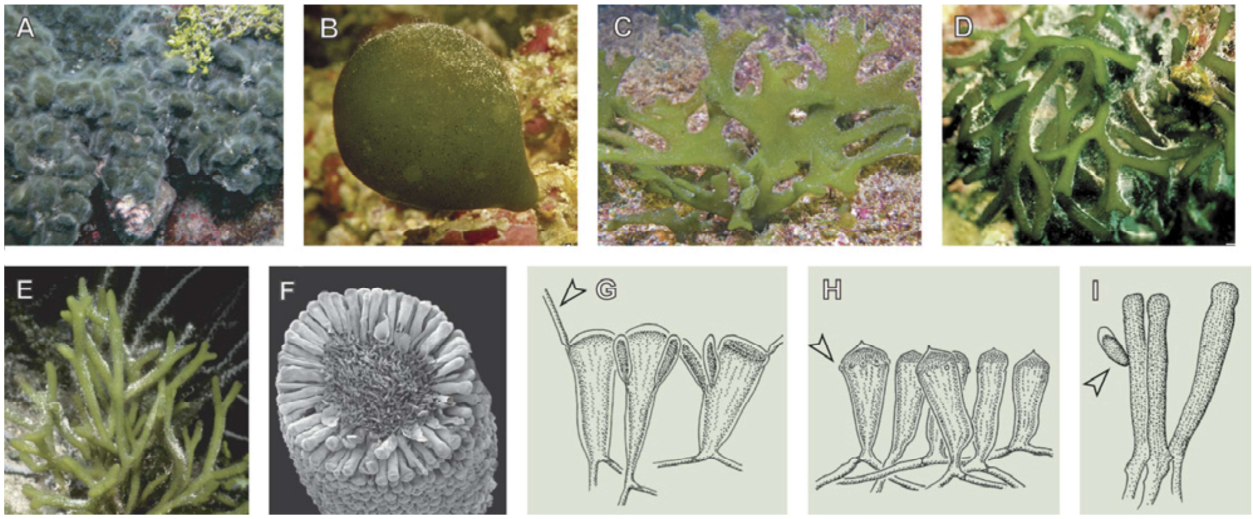


Figure 1 - Morphological diversity of *Codium* (extracted from Verbruggen *et al.*, 2007). (A) Mat-forming thallus. (B) Spherical thallus. (C) Erect thallus with flattened branches. (D) Branched thallus with a sprawling habit. (E) Erect thallus with cylindrical branches. (F) Cross-section through cylindrical branch showing the central medulla composed of a disorganized mesh of siphons, surrounded by a cortex composed of a uniform layer of utricles. (G) Club-shaped utricles with utricle hairs (arrow) and gametangia. (H) Club-shaped utricles with a pointed tip (mucron) and hair scars (arrow). (I) Cylindrical utricles with a gametangium (arrow). Utricles sizes range from 40-550 µm width and 400-6500 µm length.

3.1.6 The species *Codium elisabethae* O.C. Schmidt (1929)

First identifications, descriptions of morphology and presence of *Codium elisabethae* occurred in the Azores (Schmidt, 1929), after probable confusion with *Codium bursa* (Agardh, 1817) (Silva, personal communication).

Codium elisabethae morphology is described as a globose or subglobose perennial thallus formed of a spherical entangled network of siphonous filaments enclosing an isolated seawater volume. The thallus is moderately firm, dark green, adherent to substratum by a tuft of rhizoidal attachments. The thallus dissecting out into individual utricles or pairs of utricles, the second utricle being produced directly from the parent utricle (Guiry and Guiry, 2011); mature utricles of adult thallus subcylindrical to slightly clavate, 265-530 µm diameter, (2.5-) 3-5.5 (-6.7) mm long; apices of utricles broadly rounded but acuminate and slightly asymmetrical; utricular wall 2-3 µm thick, to 85 µm thick at apex, where it forms a more or less pronounced obtuse mucro; apical wall finely lamellate. Hair scars relatively abundant on certain utricles, forming band 350-650 µm below apex. Medullary filaments of 60-110-130 µm diameter; gametangia narrowly ellipsoidal to cylindrical, usually tapered anteriorly, of 65-130 µm diameter, 370-550 µm long, borne usually one (sometimes two, rarely three) per utricle, on pedicel 15-25 µm long, 720-840 µm below apex of utricle.

Codium elisabethae and *Codium bursa* are both similar dark green spongy balloon-like species which are indistinguishable externally (Chacana, 2002). Their thallus generally becomes centrally depreciated and more irregular with age. The two species are known up to now to differ only by their geographical ranges (see section 3.4.2) and by microscopic morphological differences in the shape and size of their utricles apex. The anatomical distinction between the 2 species concerns the apex of their utricles, which are generally

broadly rounded, asymmetrical and slightly thickened (6-25 µm thick) for *C. bursa*, whereas they are more regular, thin (85 µm thick at apex) and whith clear mucron shape for *C. elisabethae*.

3.2 Recent phylogenetic studies of the genus *Codium*

This section presents a synthetic view of most recent studies on the molecular position of the *Codium genus*, as of many of its species.

According to (Leliaert *et al.*, 2011a and 2011b), the genus *Codium* is considered to be part of the Bryopsidales (also referred to as the *Caulerpales*, *Codiales* or *Siphonales*), together with the genus *Bryopsis*, *Caulerpa* and *Halimeda*. Thus, *Bryopsidales* are considered to range in morphology from simple, branched siphons (e.g., *Bryopsis*, *Chlorodesmis*) to more complex, differentiated thalli (*Codium*, *Halimeda*, *Udotea*, *Caulerpa*). Yet two main clades are appearing in phylogenetic studies (Lam and Zechman, 2006; Verbruggen *et al.*, 2009), corresponding to the suborders Bryopsidineae (*Codiaceae*, *Bryopsidaceae*, *Derbesiaceae*) and *Halimedineae* (*Caulerpaceae*, *Rhipiliaceaea*, *Halimedaceae*, Pseudo*codiaceae* and *Udoteaceae*). Species boundaries and phylogenetic relationships within the genus *Codium* were analyzed by Verbruggen *et al.* (2007) based on plastid DNA sequences, but *Codium elisabethae* was not included in this analysis.

3.3 The genus *Codium:* scientific and economic interests

3.3.1 Original genome and active speciation

According to Silva (1992), a large part of the genus *Codium* seems to be actively speciating. Focusing on partial chloroplast DNA data, (Manhart *et al.*, 1989) showed that the gene order in the *Codium* genome bears no marked resemblance to either the "consensus" vascular plant order or to that of any green algal or bryophyte genome.

For benthic marine algae, the highest degree of intrageneric morphological diversity is exhibited by the genus *Codium* and *Caulerpa*, both of which are well represented on warm-cool transitional coasts (Silva, 1992). Both genera are excellent examples of evolutionary variations on a theme.

3.3.2 *Codium* biogeography: a model for scientific research

Within the marine green algae, there are few genera that can be used as a model for studies of speciation history, evolution and biogeography. According to Guiry and Guiry (2011), most of the *Codium* species have narrow geographic ranges and exhibit little morphological variability, while a few species range widely and exhibit complex patterns of morphological variability. According to Verbruggen *et al.* (2007), the genus *Codium* constitutes an ideal example because it is distributed through much of the world's seas, shows a wide variety of

forms and occurs in various habitats. *Codium* grows intertidally, and to at least -40m. It occurs in all marine waters except the Arctic and Southern Oceans. The largest numbers of species are found in floras that are transitional between temperate and subtropical, namely Japan (19), South Africa (19), Australia (l8), and California-Mexico (12). Each of these floras includes a member of most sections of the genus, indicating an ancient dispersal of the progenitors. Because of lack of calcification, there is no fossil record of the genus.

For the last two decades, *Codium* has been in the public and scientific spotlight because of the invasive, bloom forming nature of certain species (Verbruggen *et al.*, 2007. *Codium fragile* subspecies *tomentosoides* is the most invasive seaweed in the world, being unintentionally spread around the globe with cultured shellfish (Trowbridge, 1998). Another species, *C. isthmocladum*, forms harmful blooms on South Florida reefs in conjunction with increased eutrophication (Lapointe *et al.*, 2005). Both species can damage shellfish beds and perturb native communities and massive amounts of rotting thalli can smother shores (Verbruggen *et al.*, 2007). Since 2004, several massive blooms of *Codium parvulum*, originally described from the Red Sea, have been newly reported for the Mediterranean in the northern shores of Israël (Israel *et al.*, 2010). Provan *et al.* (2005 and 2008) employed a combination of plastid microsatellite markers and DNA sequence data from three regions of the plastid genome to trace the invasive history of the green alga *Codium fragile* ssp *tomentosoides* ("oyster thief"). These invasive populations displayed a high level of geographical structuring of haplotypes, with one haplotype localized in the Mediterranean and the other found in Northwest Atlantic, northern European and South Pacific populations. All three of the regions of the genome of *Codium fragile* ssp *tomentosoides* sequenced by Provan *et al.* (2005) revealed intraspecific and intrapopulation variation, as in other *Codium* species (Provan, unpublished).

Tyberghein *et al.* (2011) illustrated the high potential of their new data package Bio-ORACLE (ocean raster's for analysis of climate and environment) for species distribution modelling of shallow-water marine organisms by demonstrating its high predictive power for the distribution model of *Codium fragile* subsp. *fragile*.

3.3.3 Life cycle and reproduction

Despite the ubiquity of the *Codium* genus, relatively little is known about its biology in general and reproduction in particular (Guiry and Guiry, 2011; Miravalles *et al.*, 2011). Tanner (1981) stated that life histories of most of the species and genera of the *Bryopsidales* Order (then called *Caulerpales*) were yet either unknown or incompletely studied, and considered essential that future studies would include information regarding the development of zygotes and detailed cytological evidence. *Codium* reproductive behavior varies among species and even the same species may have different reproductive strategies in different locations. Guiry and Guiry (2011) generalized a general life history (outlined below) from fragmentary studies. *Codium* genus is initially presented as having a gametic type life cycle (Williams, 1925) with single diploid vegetative phase (Chapman, 1964; Lee, 1999; Van den Hoek et al., 1995) as it is generally the case for *Codium fragile* spp. *tomentosoides* from the Pacific coast and *Codium fragile* spp. *novae-zelandiae* (Miravalles *et al.*, 2011; Miravalles, personal communication). As illustrated in figure 2, gametangia are produced laterally by utricles, each with a basal plug above a short pedicel. They are fusiform to ovoid with a content cleaving into biflagellate gametes following the meiosis. Gametes are extruded in a gelatinous mass through apical rupture. Male gametes contain only one or two chloroplasts, while female gametes are several times larger and with numerous chloroplasts. The two types

of gametes can be produced on the same thallus (monoecious) or more often on different thalli (dioecious). For instance, *Codium fragile* can either be unisexual or bisexual, the bisexual thalli possibly resulting from the interweaving of filaments from two or more zygotes (Arasaki *et al.*, 1955). Zygote develops into amorphous prostrate vesicle that produces erect elongate vesicles; these in turn initiate primary utricle-producing siphons which eventually consolidate into a multiaxial thallus. In addition to this general life cycle depicting a diploid macroscopic thallus, haploid thallus also exist as asexual reproduction occurs by parthenogenesis of a single gamete type, as mentioned for *C. vermilara*, *C. decorticatum* and some populations of *C. fragile* subsp. *novae-zelandiae* from the southwest atlantic coasts by Miravalles (personal communication) and for *Codium tomentosum* by Dangeard and Parriaud (1956). In the northeast Atlantic, populations of *C. fragile* ssp. *tomentosoides* have been also observed to release biflagellate swarmers that germinated directly (Churchill and Moeller, 1972; Ramus 1972). Female gametes are generally mentioned as the one involved in parthenogenesis, but Miravalles *et al.* (2003) suggested that agamic germination of male gametes would be the only asexual reproduction of Argentinian populations of *Codium decorticatum*, as these populations were never observed to produce any female gametes. Asexual reproduction is also reported by fragmentation, or by the cutting off of modified aborted gametangia. In some genera vegetative reproduction may be important (Borden and Stein, 1969; Fralick and Mathieson, 1972).

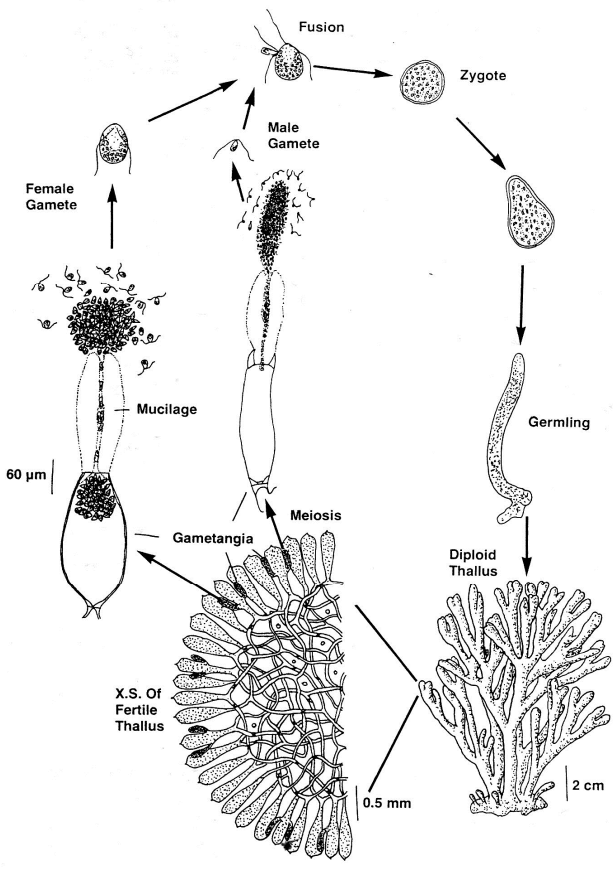


Figure 2 - *Codium* genus typical life cycle (Lee, 1999).

Focusing on examples of *Caulerpa* and *Codium*, Vroom and Smith (2001) underlined the interesting complexity and efficiency of reproduction propagation modes, notably for species

of the *Codium* genus which is generally sensitive to environmental conditions and grow in precise optimal conditions.

Biological invasions are acknowledged to be among the most severe threats to biological diversity in terrestrial and marine environments (Grosholz, 2002). Together with the physical and biological characteristics of the habitat, the life-history traits of the invader have been indicated as key determinants of successful invasions. By modifying habitat conditions, global changes might trigger or affect invasions. To address this issue, the variability of life history and reproduction of an organism in regard to environmental conditions has to be precisely described. Bulleri *et al.* (2007) studied the temporal dynamics, the spatial scales of variation and the effects of wave exposure on the abundance of reproductive structures of *C. fragile*. They found gametangia from June to September and suggested that the provision of sheltered rocky habitats has been crucial for the establishment of *C. fragile* in the northern Adriatic Sea.

3.3.4 Largest unicellular organism and variable growth forms

The *Codium* macroalgae are among the largest unicellular organisms (Pedroche *et al.*, 2002), together with some species of the genus *Caulerpa*, reaching up to 3 m long (Meinesz, personal communication). Variable growth forms have been observed by Blunden *et al.* (1989) for the same species (*Codium fragile* subspecies *tomentosoides*) on floating structures, indicating that the life cycle might be more complex than supposed, maybe favouring survival or dispersion according to conditions.

3.3.5 *Codium* growth patterns in response to environmental factors

Information concerning seasonal patterns of growth, reproduction, and productivity of *Codium* are quiete rare. One very complete *in situ* and laboratory study concerned *Codium fragile* spp. *tomentosoides* (van Goor) Silva populations monitored at 3 locations in Rhode Island by Hanisak (1979). Maximal growth occurred during the summer and was more significantly correlated with temperature than any other factor measured in this study. Multiple correlation models suggested an interaction between temperature, irradiance, and available nitrogen. Maximal reproduction occurred in late summer and early fall. The maximal productivity, based on harvested quadrats, was 2.10 g dry weight.m^{-2}.day^{-1}. A large amount (up to 87.3%) of the annual production entered the detrital food chain during the winter by fragmentation of the thallus. Culture studies examined the effects of temperature (6° to 30°C), irradiance (7 to 140 µE.m^{-2}.sec^{-1}), day length (8 h light: 16 h dark to 24 h light: 0 h dark) and salinity (6 to 48) on growth. Differentiated thalli grew over a broad range of experimental conditions, with maximal growth at 24°C, 24 to 30 S, a minimal irradiance of 28 µE.m^{-2}.sec^{-1}, and 16 h day length. The effect of increasing daylength was due to increased total daily irradiance rather than to a true photoperiodic effect. Undifferentiated sporelings survived and grew in a narrower range of environmental conditions than thalli. *C. fragile* spp. *tomentosoides* grew equally well with nitrate, nitrite, ammonium, and urea as a nitrogen source. The addition of $NaHCO_3$ stimulated growth at levels of 2.4 to 4.8 mM, suggesting an inorganic carbon limitation in static cultures. This study supports the hypothesis that the *in situ* seasonal growth pattern of *C. fragile* spp. *tomentosoides* is primarily due to the interaction of temperature and irradiance.

3.3.6 Biochemical properties

Many interesting biochemical properties are known for the *Codium* genus: larvicids, anticoagulant, anticancerigenic, antifungal properties. Various *Codium* species are used in folk medicine as vermifuge, especially against *Ascaris lumbricoides* (Hoppe and al., 1979). Rogers and Fanglu (1991) studied lectins from *Codium* species. Anticoagulant activities were studied for *Codium bursa* and *C. vermilara* (Jurd *et al.*, 1992). Antiplasmodial potential and leishmanicidal activity were demonstrated for *Codium bursa* and other algae by Süzgeç-Selçuk *et al.* (2011). Bactericidal activity were demonstrated from *Codium iyengarii* (Borgesen) collected from Karachi coast of Arabian Sea (Shaiq Ali, 2002), as for *Codium fragile* (Koz *et al.*, 2009). *Codium* has served as a model organism for studies of algal physiology and ecophysiology, heavy metal accumulation and bioactive compounds (Mayer *et al.* 2007; Trowbridge, 1998). Because of its ready availability throughout the world, *Codium* was often included in comparative biochemical and physiological studies, but resulting still in many unconsolidated data (Guiry and Guiry, 2011).

3.3.7 Natural predators and human alimentary uses

Codium species were observed to be grazed by sea urchins (*Stronglyocentrotus droebachiensis*) (Lyons and Scheibling, 2007), sea slug mollusk (Gallop *et al.*, 1980), crabs (Saisho *et al.*, 1983; Bourdeau and O'Connor, 2003). *Codium* species are used as food for cultured abalone shell (*Haliotis*) (Uki, 1984) but also consumed by humans (Verbruggen *et al.*, 2007). Exploitation of wild *Codium* population (wild harvesting) for human food reaches up to 50 Tons/year in North Korea and 50 Tons/year in South Korea. *Codium* species are also consumed elsewhere, mainly by communities of ‘sea veget eaters’ and by communities interested by ‘well-being effects’. According to Dawson (1966), the Hawaiians developed the most diversified dietary use of seaweeds with 75 species used regularly as food. Among them, an intertidal *Codium* species is today sold in Hawaiian markets under the name of Wawaeiole. Like the green alga *Ulva*, it is cooked and eaten in soups. The functional and nutritional values of the Chilean *Codium fragile* were recently studied by Ortiz *et al.* (2009), together with the other macroalgae *Gracilaria chilensis* and *Macrocystis pyrifera*.

3.4 *Codium elisabethae* and *Codium bursa*: ecological interests

3.4.1 An isolated seawater micro ecosystem

Several studies quantified the ecology of *Codium bursa* (Geertz-Hansen *et al.* 1994, Vaqué *et al.* 1994; Vidondo and Duarte 1995; Vidondo and Duarte 1998). These studies focused on relatively shallow population living in a sheltered bay in Cala Jonquet, on the Spanish Mediterranean coast.

The functional implications of the form of *Codium bursa* was studied by Geertz-Hansen *et al.* (1994). The internal seawater volume of *C. bursa* was demonstrated to constitute a small but very specific isolated ecosystem enriched in micro-heterotrophs (Vaqué *et al.*, 1994) and in nutrients (Vidondo and Duarte, 1995), as compared to seawater. The nutrient concentration ranges measured in the site studied by Vidondo and Duarte (1995) and Geertz-Hansen *et al.* (1994) were of 0.33 – 4 µM/l for nitrates; 1.12 µM/l for ammonium and 0.04 – 0.84 µM/l for phosphates.

Vidondo and Duarte (1995) showed that for their studied population, nutrient concentrations in the water enclosed within large *Codium bursa* individuals followed a pattern parallel to that of ambient nutrient concentrations ($r = 0.76$ and $r = 0.73$, $p < 0.005$, for phosphate and nitrates, respectively). Yet, dissolved inorganic nitrogen (DIN) concentrations in the internal water largely exceeded (by about 10 fold) ambient concentrations, except from July to October, when they were similar. In contrast, phosphate concentrations in the internal water were substantially lower than ambient concentrations, except in winter when internal concentration exceeded ambient ones. These results indicate that the water in the internal lumen may represent an important reservoir for nitrogen, but not for phosphore, and points to a greater demand for P, relative to N, by the macroalga. Globally, the nutrient concentration factor observed by these authors ranged from 5 to 10 for the measured nutrients.

Based on growth rate measurements, *Codium bursa* was announced to be a long living green algae, with a lifespan estimate reaching 15 years (Vidondo and Duarte, 1998).

Relatively little is known concerning the ecology of *C. elisabethae* as compared to *C. bursa.* The rare work concerning *Codium elisabethae* remained short morphological descriptions and only rare studies focused on a more precise evaluation of the habitat and affinities of *C. elisabethae* (Tempera, 2009) or focused on the description of the variability of population density (Neto, 1997 and 2000). This last work exploited relatively small samples observed randomly within shallow water population, and did not conclude in any seasonal trends in growth rate (Neto, 1997).

3.4.2 Biogeography

Codium bursa C. Agardh was first described in 1817. Its distribution range has since then been identified by numerous authors (Guiry and Guiry, 2011) as covering the whole Mediterranean sea together with a long stretch of the eastern Atlantic coasts from the British Isles till north Africa, the whole Canarian arquipelago, and New Zealand. A plethora of references supports the presence of the globose macroalga identified as *Codium bursa* for the following specific regions: Ireland (Antrim, Donegal, Down), Adriatic, Balearic Islands, Britain, Corsica, France, Greece, Isla de Alborán, Italy, Malta, Spain, Turkey, Azores, Canary Islands, Algeria, Egypt, Libya, Morocco, Tunisia, Israël, Levant states, New Zealand. We refer to Guiry and Guiry (2011) for the details of literature sources.

First identifications, descriptions of morphology and presence of *Codium elisabethae* occurred more than 100 years later, in the Azores (Schmidt, 1929), after probable confusion with *Codium bursa* (Silva, personal communication), as *C. bursa* was never confirmed in the Azores. Later, *Codium elisabethae* was also identified in Madeira (Levring, 1974), in the Salvage Islands (Parente *et al.* 2000), and up to now in the most northern and eastern point of the Canarian Islands, Lanzarote (Chacana, 2002). Such Macaronesian distribution of *Codium elisabethae* have been mentioned by other studies, in the Azores (Neto, 1994, 1997 and 2000; Schmidt, 1931; Tittley and Neto, 1994), the Canary Islands (Chacana *et al.*, 2003; Gil-Rodríguez *et al.* 2003; Haroun *et al.* 2002; John *et al.* 2004), Madeira (Audiffred and Prud'homme van Reine, 1985; John *et al.* 2004; Neto, Cravo and Haroun 2001) and the Salvage Islands (John *et al.* 2004).

The synthetic distribution of *Codium elisabethae* and *Codium bursa* listed in the literature is illustrated within the North-East Atlantic and Western Mediterranean in figure 3. Concerning Codium bursa, it is probably more continuous than actually listed along the Atlantic European coasts, but further confirmations are required. This distribution is projected over a background SST (Sea Surface Temperature; field extracted from the Ocean Color web page (Feldman and McClain, 2011) as a mission composite constructed by using all the measurements by the satellite Aqua from July 2002 to June 2011. It shows that *Codium elisabethae* is found in locations where SST annual average is close to 20°C, whereas the distribution of *Codium bursa* covers a wider range of annual average SST (13-23°C).

In Lanzarote, the only known overlap between both algae's distribution ranges, Chacana (2002) suggested that their habitat might be distinct in depth as it is not known whether there is any site where the two species grow side by side.

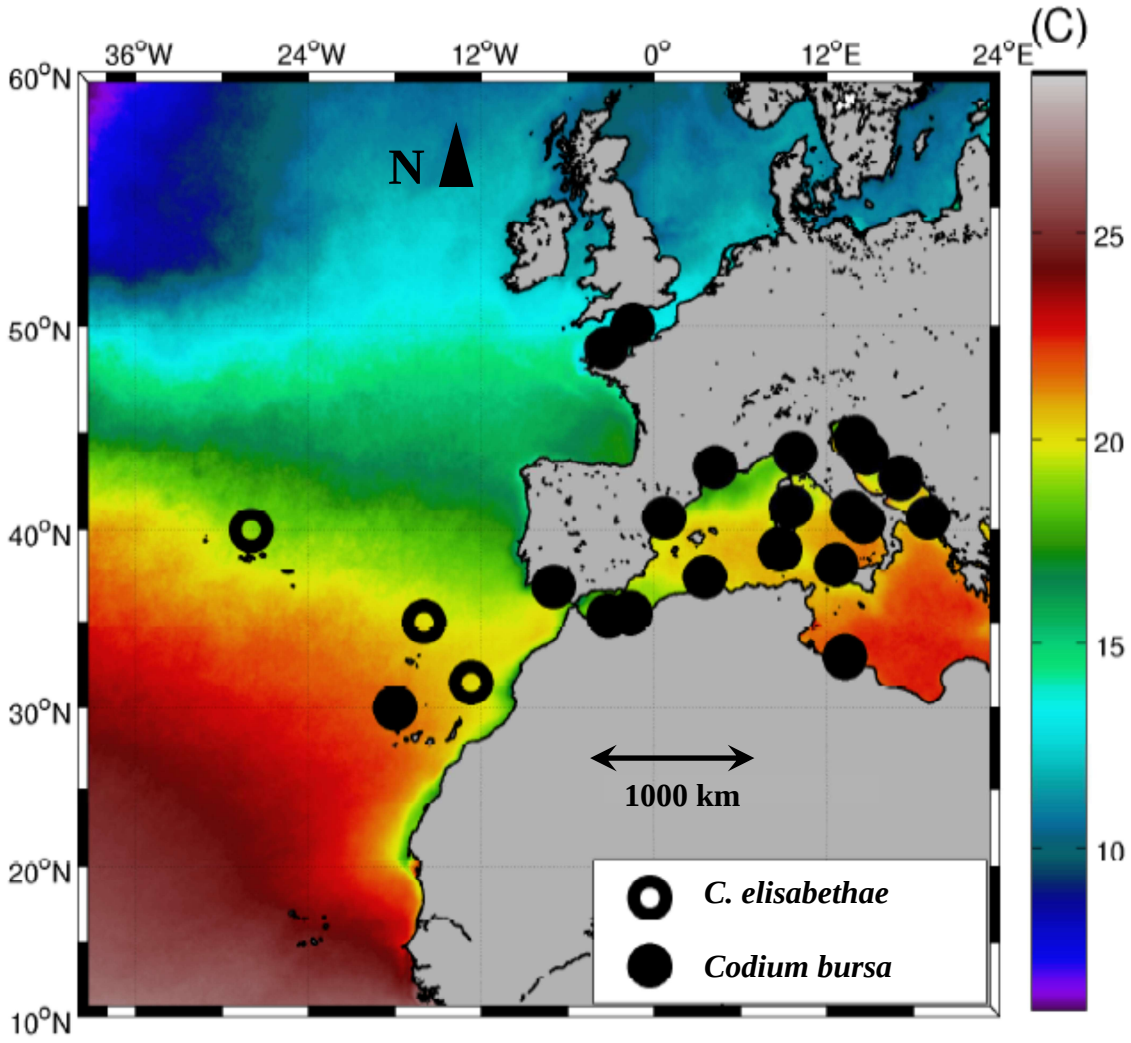


Figure 3 - Synthetic distribution of *Codium elisabethae* and *Codium bursa within the North-East Atlantic and Western Mediterranean*, showing narrow range of annual mean temperature for the habitat of *C. elisabethae* (adapted from Chacana 2002, over the 2002-2011 average SST field (°C) from Feldman and McClain, 2011).

3.4.3 Habitat types

The EUNIS Database is the European Nature Information System, developed and managed by the European Topic Centre on Biological Diversity (ETC/BD in Paris) for the European Environment Agency (EEA) and the European Environmental Information Observation Network (Eionet). Habitat types of both *Codium elisabethae* and *Codium bursa* were earlier mentioned by the EUNIS habitat classification to correspond to the habitats « Infralittoral rocks in wave and tide-sheltered conditions », or « Sheltered infralittoral bedrock, with *Halopteris filicina* and coralline crusts ». It has now been reviewed (EUNIS, 2011) to habitat type « Infralittoral rock and other hard substrata » (EUNIS code A3).

Guiry and Guiry (2011) mention an habitat affinity for *Codium elisabethae* ranging from Intertidal pools down to 20 m, Neto (1997) reported their presence from 3 down to 15 meters in São-Miguel island (Azores), whereas Tempera (2009) described habitats depths ranging from few meters deep till 40 meters around Faial and Pico islands (Azores), as described in Chapter 2. In the European coasts, *Codium bursa* is mentioned to be found from 1 to 45 meters deep, and was even signaled down to 90 meters (Cabioc'h et al., 2006).

At shallow white hydrothermal vents, the green alga *Codium elisabethae* was the closest macro-alga to the vents and was found at distances of 0–140 cm from the vent outlets, in warm and acid waters (T=26-42°C; pH=5), highly charged in H_2S, CH_4, H_2, Pb and CO (Cardigos *et al.*, 2005). It is interesting to note this highest tolerance to specific hydrothermal vent conditions for a species known as an endemic to the Macaronesian islands, all of which are from volcanic origin.

Although it is not known if *Codium elisabethae* and *Codium bursa* are capable of hybridizing, Chacana (2002) raised the question of the nature of a potential ecological barrier that might prevent the interbreeding and merging of the two species in the only known overlap of their biogeographical ranges. Before addressing such hypothesis, molecular data would be needed to confirm species boundaries and to study potential hybridization (Leliaert, personal communication).

In parallel, precise description of habitat ranges and identification of potential differences in ecology is also a very interesting question remaining to be solved for these two species. Their relative biogeography should investigated in regard to the general knowledge accumulated concerning the phytogeographic distribution of macroalgae in the North Atlantic Ocean (Van den Hoek, 1982a), the role of temperatures in their life histories (Van den Hoek, 1982b), and notions of long range dispersal (Van den Hoek, 1987).

3.4.4 Interesting perspectives

New climatologies are currently established with modern geostatistical tools over several parts of this global distribution range, as by Troupin *et al.* (2010) over the North-East Atlantic, Sirjacobs *et al.* (2011) over the English Channel, and by Troupin *et al.* (2009) and Sirjacobs *et al.* (2009) over the western Mediterranean. As demonstrated at global scale by Tyberghein *et al.* (2011), such type of improved climatologies are of high interest to support refined and complementary investigations on the environmental factors potentially explaining the present distribution ranges of marine organisms. This could be applied to the case of *Codium elisabethae* and *Codium bursa* macroalgae at large scale. More local scales distributions could be addressed based on combined spatialized databases approaches as illustrated from coastal scale (Tempera, 2009) to regional scale (Pauly, 2011), integrating population dynamics parameters revealed by Vidondo *et al.* (1995 and 1998) and by the present research approach.

3.5 References

Adl, S.M., Simpson, A.G.B., Farmer, M.A., Andersen, R.A., Anderson, O.R., Barta, J.R., Bowser, S.S., Brugerolle, G., Fensome, R.A., Fredericq, S., James, T.Y., Karpov, S., Kugrens, P., Krug, J., Lane, C.E., Lewis, L.A., Lodge, J., Lynn, D.H., Mann, D.G., McCourt, R.M., Mendoza, L., Moestrup, O., Mozley-Standridge, S.E., Nerad, T.A., Shearer, C.A., Smirnov, A.V., Spiegel, F.W. and Taylor, M.F., 2005. The new higher level classification of eukaryotes with emphasis on the taxonomy of protists. Journal of Eukaryotic Microbiology, 52: 399-451.

Agardh, C.A., 1817. Synopsis algarum Scandinaviae, adjecta dispositione universali algarum. *Lundae [Lund]: ex officina Berlingiana.* pp. [i]-xl, [1]-135.

Arasaki, S., Tokuda, H. and Fujiama, K., 1955. The reproduction and morphogeny in *Codium fragile*. Botanical Magazine Tokyo, 69: 39-45.

Audiffred, P.A.J. and Prud'homme van Reine, W.F., 1985. Marine algae of Ilha do Porto Santo and Deserta Grande (Madeira Archipelago) (CANCAP project Contribution No. 40). Boletim do Museu Municipal do Funchal, 37(166): 20-51.

Bessey, C.E., 1907. A synopsis of plant phyla. Nebraska University Studies, 7: 275-373.

Blackman, F.F. and Tansley, A.G., 1902. A revision of the classification of the green algae. New Phytologist, 1: 17-24, 47-48, 67-72, 89-96, 114-120, 133-144, 163-168, 189-192, 213-220, 238-244.
doi: 10.1111/j.1469-8137.1902.tb06576.x

Blunden, G., Fletcher, R.L., Smith, B.E., Rogers, D.J. and Fish, B.C., 1989. An unusual growth form of *Codium fragile* (Sur.) Hariot subspecies *tomentosoides* (Goor) Silva found on floating structures in the Solent. British Phycological Journal, 24: 299.

Borden, C.A. and Stein, J.R., 1969. Reproduction and early development in *Codium fragile* (Suringar) Hariot: *Chlorophyceae*. Phycologia, 8: 91-99.

Bourdeau, P.E. and O'Connor, N.J., 2003. Predation by the nonindigenous Asian shore crab *Hemigrapsus sanguineus* on macroalgae and molluscs. Northeastern Naturalist, 10(3): 319-334. doi: http://dx.doi.org/10.1656/1092-6194(2003)010[0319:PBTNAS]2.0.CO;2

Brodie, J., Maggs, C.A. and John, D.M., 2007. Green seaweeds of Britain and Ireland. London: British Phycological Society. pp. [i-v], vi-xii, 1-242.

Bulleri, F., Branca, M.G., Abbiati, M. and Airoldi, L., 2007. Development of reproductive structures in the introduced green alga, *Codium fragile* ssp. *tomentosoides*, in the northern Adriatic Sea. European Journal of Phycology, 42(2): 137-144.
doi: 10.1080/09670260701231243

Cabioc'h, J., Floc'h, J.Y., Le Toquin, A., Boudouresque, C.F., Meinesz, A. and Verlaque, M., 2006. Guide des algues des mers d'Europe. Delachaux et Niestlé SA, Paris, pp 272.

Cardigos, F., Colaço, A., Dando, P.R., Ávila, S.P., Sarradin, P.-M., Tempera, F., Conceição, P., Pascoal, A. and Santos, R.S., 2005. Shallow water hydrothermal vent field fluids and communities of the D. João de Castro Seamount (Azores). Chemical Geology, 224: 153-168.

Chacana, M., 2002. *Codium elisabethae* O.C. Schmidt, newly recorded from the Canary Islands. Constancea 83-17. University of California Electronic Publications in Botany. http://ucjeps.berkeley.edu/constancea/83/chacana/C_*elisabethae*.html.

Chacana, M., Silva, P.C., Pedroche, F.F. and Gil-Rodríguez, M.C., 2003. *Codium profundum* y *C. guineense*: nuevas citas para las islas Canarias y observaciones en *C. elisabethae* (Chlorophycota). Vieraea, 31: 267-280.

Chapman, V.J., 1964. The Chlorophyta. Oceanography and Marine Biology: an annual review, 2: 193-228.

Churchill, A.C. and Moeller, H.W., 1972. Seasonal patterns of reproduction in New York populations of *Codium fragile* (Sur.) Hariot subsp. *tomentosoides* (van Goor) Silva. Journal of Phycology, 8: 147-152.

Dangeard, P. and Parriaud, H., 1956. Sur quelques cas de développement apogamique chez deux espèces de *Codium* de la region du sudouest. Comptes rendus Hebdomadaires des Séances de l'Académie des Sciences, Paris 243: 1981-1983.

Dawson, E.Y., 1966. Marine Botany, an introduction. Holt, Rinehart and Winston, New York, 371 pp.

EUNIS, 2011. http://eunis.eea.europa.eu/; as searched on the 15/08/11.

Feldmann, J., 1954. Sur la classification des Chlorophycées siphonées. VIII Congrès International de Botanique Paris. Rapp. and Comm., sect. 17: 96–98.

Feldman, G.C. and McClain, C.R., 2011. Ocean Color Web, http://oceancolor.gsfc.nasa.gov/. NASA Goddard Space Flight Center, Eds. Kuring, N., Bailey, S.W., Franz, B.F., Meister, G., Werdell, P.J., Eplee, R.E., accessed August 19, 2011.

Fralick, R.A. and Mathieson, A.C., 1972. Winter fragmentation of *Codium fragile* (Suringar) Hariot spp. *tomentosoides* (van Goor) Silva (Chlorophyceae, Siphonales) in new England. Phycologia, 11: 67-70.

Gallop, A., Bartrop, J., and Smith, D.C., 1980. The biology of chloroplast acquisition by *Elysia viridis*. Proceedings of the Royal Society of London, Biological Sciences, 207(1168): 335-349. doi: 10.1098/rspb.1980.0027

Geertz-Hansen, O., Enriquez, S., Duarte, C. M., Agusti, S., Vaque, D. and Vidondo, B., 1994. Functional implications of the form of *Codium bursa*, a balloon-like Mediterranean macroalga. Marine Ecology Progress Series, 108: 153-160. http://www.int-res.com/articles/meps/108/m108p153.pdf

Gil-Rodríguez, M.C., Haroun, R., Ojeda Rodríguez, A., Berecibar Zugasti, E., Domínguez Santana, P. and Herrera Morán, B., 2003. Proctoctista. In: Lista de especies marinas de Canarias (algas, hongos, plantas y animales). (Moro, L., Martín, J.L., Garrido, M.J. and Izquierdo, I. Eds), Las Palmas: Consejería de Política Territorial y Medio Ambiente del Gobierno de Canarias, pp. 5-30.

Grosholz, E., 2002. Ecological and evolutionary consequences of coastal invasions. Trends in Ecology and Evolution 171: 22–27.

Guiry, M.D. and Guiry, G.M., 2011. AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. http://www.algaebase.org ; searched on 25 August 2011.

Hanisak, M.D., 1979. Growth patterns of *Codium fragile* ssp. *tomentosoides* in response to temperature, irradiance, salinity, and nitrogen source. Marine Biology, 50(4): 319-332. doi: 10.1007/BF00387009

Haroun, R.J., Gil-Rodríguez, M.C., Díaz de Castro, J. and Prud'homme van Reine, W.F., 2002. A checklist of the marine plants from the Canary Islands (central eastern Atlantic Ocean). Botanica Marina, 45: 139-169.

Hillis-Colinvaux, L. 1984. Systematics of the Siphonales. In: Systematics of the green algae. Irvine, D. E. G. & John, D. M. Eds., Academic Press, London, 271-291.

Hoppe, H.A., Levring, T. and Tanaka, Y., 1979. Marine algae in Pharmaceutical science. Ed. Hoppe, H.A., W. de Grutyer – Berlin - New York, 807 pp.

Israel, A., Einav, R., Silva, P.C., Paz, G., Chacana, M.E. and Douek, J., 2010. First report of the seaweed *Codium parvulum* (Chlorophyta) in Mediterranean waters: recent blooms on the northern shores of Israël. Phycologia, 49: 107-112. doi: 10.2216/09-28.1.

John, D.M., Prud'homme van Reine, W.F., Lawson, G.W., Kostermans, T.B. and Price, J.H., 2004. A taxonomic and geographical catalogue of the seaweeds of the western coast of Africa and adjacent islands. Beihefte zur Nova Hedwigia, 127: 1-339.

Jurd, K., Rogers, D.J. and McLellan, D.S. 1992. Anticoagulant activities from *Codium bursa* and *C. vermilara*. British Phycological Journal, 27: 95.

Koz, F.F.Y., Yavasoglu, N.U.K., Demrel, Z., Sukatar, A. and Ozdemr, G., 2009. Antioxidant and antimicrobial activities of *Codium fragile* (Suringar) Hariot (Chlorophyta) essential oil and extracts. Asian Journal of Chemistry, 21(2): 1197-1209.

Kützing, F.T., 1843. Phycologia generalis oder Anatomie, Physiologie und Systemkunde der Tange... Mit 80 farbig gedruckten Tafeln, gezeichnet und gravirt vom Verfasser. Leipzig: F.A. Brockhaus, pp. [part 1]: [i]-xxxii, [1]-142, [part 2:] 143-458.

Lam, D. W. and Zechman, F. W. 2006. Phylogenetic analyses of the Bryopsidales (Ulvophyceae, Chlorophyta) based on Rubisco large subunit gene sequences. Journal of Phycology, 42: 669-678.

Lapointe, B.E., Barile, P.J., Littler, M.M., Littler, D.S., Bedford, B.J. and Gasque, C., 2005. Macroalgal blooms on southeast Florida coral reefs. I. Nutrient stoichiometry of the invasive green alga *Codium isthmocladum* in the wider Caribbean indicates nutrient enrichment. Harmful Algae, 4: 1092–1105.

Lee, R.E., 1999. Phycology. Cambridge University Press, UK. 3rd Edition, 614 pp.

Leliaert, F., Verbruggen, H. and Zechman, F.W., 2011a. Into the deep: New discoveries at the base of the green plant phylogeny. BioEssays, 33: 683–692. doi: 10.1002/bies.201100035

Leliaert F., Smith D.R., Moreau H., Herron M., Verbruggen H., Delwiche C.F. and De Clerck O. 2011b. Phylogeny and molecular evolution of the green algae. Critical Reviews in Plant Sciences (in press).

Levring, T., 1974. The marine algae of the Archipelago of Madeira. Boletim Museo Municipale Funchal, 28: 5-111.

López-Bautista, J.M., Waters, D.A. and Chapman, R.L., 2003. Phragmoplastin, green algae and the evolution of cytokinesis. International Journal of Systematic and Evolutionary Microbiology, 53: 1715-1718.
doi: 10.1099/ijs.0.02561-0

Lyons, D.A. and Scheibling, R.E., 2007. Differences in somatic and gonadic growth of sea urchins (*Stronglyocentrotus droebachiensis*) fed kelp (*Laminaria longicruris*) or the invasive alga *Codium fragile* ssp. *tomentosoides* are related to energy acquisition. Marine Biology, 152: 285-295.
doi: 10.1007/s00227-007-0682-4

Manhart, J.R., Kelly, K., Dudock, B.S. and Palmer, J.D., 1989. Unusual characteristics of *Codium fragile* chloroplast DNA revealed by physical and gene mapping. Molecular and General Genetics, 216(2-3): 417-421.
doi: 10.1007/BF00334385

Mayer, A. M.S., Rodriguez, A. D., Berlinck, R. G.S. and Hamann, M. T., 2007. Marine pharmacology in 2003-4: Marine compounds with anthelmintic antibacterial, anticoagulant, antifungal, anti-inflammatory, antimalarial, antiplatelet, antiprotozoal, antituberculosis, and antiviral activities; affecting the cardiovascular, immune and nervous systems, and other miscellaneous mechanisms of action, Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology, 145(4): 553-581.
doi: 10.1016/j.cbpc.2007.01.015.

Meinesz, A., 1980. Contribution à l'étude des Caulerpales (Chlorophytes). Avec une mention particulière aux espèces de la Méditerranée occidentale. PhD Thesis, University of Nice, France, 27 May 1980, 262 pp.

Miravalles, A.B., Leonardi, P.I. and Cáceres, E.J., 2003. Fine structure of the utricles and gametogenesis of *Codium decorticatum* (Caulerpales, Chlorophyta). Hydrobiologica, 13: 1-8.

Miravalles, A.B., Leonardi, P.I. and Cáceres, E.J., 2011. Male gametogenesis ultrastructure of *Codium fragile* subsp. *novae-zelandiae* (Bryopsidophyceae, Chlorophyta). Phycologia, 50 (4): 370–378.
doi: 10.2216/10-25.1

Neto, A.I., 1994. Checklist of the benthic marine macroalgae of the Azores. Arquipélago. Ciências Biológicas e Marinhas, 12A: 15-34.

Neto, A.I., 1997. Studies on Algal Communities de São Miguel, Azores, PhD thesis in Marine Biology, University de the Azores, Ponta Delgada, unpublished.

Neto, A.I., 2000. Observations on the biology and ecology of selected macroalgae from the littoral of São Miguel (Azores). Botanica Marina, 43: 483-498.

Neto, A.I., Cravo, D.C. and Haroun, R.T., 2001. Checklist of the benthic marine plants of the Madeira Archipelago. Botanica Marina, 44: 391-414.

Ortiz, J., Uquiche, E., Robert, P., Romero, N., Quitral, V. and Llanten, C., 2009. Functional and nutritional value of the Chilean seaweeds *Codium fragile*, *Gracilaria chilensis* and *Macrocystis pyrifera*. European Journal of Lipid Science and Technology, 111(4): 320-327.

Parente, M.I., Gil-Rodríguez, M.C., Haroun, R.J., Neto, A.I., de Smedt, G., Hernández-González, C.L. and Berecibar Zugasti, E., 2000. Flora marina de las Ilhas Selvagens: resultados preliminares de la expedición "Macronesia 2000". Revista de la Academia Canaria de Ciencias, 12(3-4): 9-20.

Pascher, A., 1914. Über Flagellaten und Algen. Ber. Dtsch. Bot. Ges., 32: 136-160.

Pauly, K., 2011. GIS-based environmental analysis, remote sensing and niche modelling of seaweed communities. Doctoral thesis. July 4, 2011. Ghent University. 222 pp.

Pedroche, F.F., Silva, P. and Chacana, M., 2002. El género *Codium* (*Codiaceae*, Chlorophyta) en el Pacífico de México. En: Sentís and Dreckmann (eds). Monografías Ficológicas 2002. Universidad Autónoma Metropolitana-Iztapalapa. México D.F.: 11-74.

Provan, J., Murphy, S. and Maggs, C.A., 2005, Tracking the invasive history of the green alga *Codium* fragile ssp. tomentosoides. Molecular Ecology, 14: 189-194.

Provan, J., Booth, D., Todd, N.P., Beatty, G.E. and Maggs, C.A., 2008. Tracking biological invasions in space and time: elucidating the invasive history of the green alga *Codium* fragile using old DNA. Diversity and Distributions, 14(2): 343-354.

Ramus, J., 1972. Differentiation of the green alga *Codium fragile*. American Journal of Botany, 59: 478-482.

Rogers, D.J. and Fanglu, H., 1991. Lectins from *Codium* species. British Phycological Journal, 26: 95-96.

Saisho, T., Noguchi, T., Koyama, K., Uzu, A., Kikuta, T. and Hashimoto, K., 1983. Examination of stomach content in Xanthid crabs. Bulletin of the Japanese Society of Scientific Fisheries, 49(6): 939-947.

Schaffner, J.H., 1922. The classification of plants XII. Ohio Journal of Science, 22: 129-139.

Schmidt, O.C., 1929. Beiträge zur kenntnis der meeresalgen der Azoren. I. Hedwigia, 69: 95-113.

Schmidt, O.C., 1931. Die marine vegetation der Azoren in ihren grundzügen dargestellt. Bibliotheca Botanica, 25(102): viii + 1-116.

Shaiq Ali, M., Saleem, M., Yamdagni, R. and Ashfaq Ali, M., 2002. Steroid and antibacterial steroidal glycosides from marine green alga *Codium Iyengarii* Borgesen. Natural Product Research: Formerly Natural Product Letters, 16 (6): 407-413.
doi: 10.1080/10575630290034249

Silva, P., 1980. Names of classes and families of living algae: with special reference to their use in the Index *Nominum Genericorum* (Plantarum). Regnum Vegetabile, 103: 1-156.

Silva. P., 1992. Geographic patterns of diversity in benthic marine algae. Pacific Science, 46(4): 429-437.

Sirjacobs, D., Lenartz, F., Troupin, C., Alvera, A., Barth, A., Ouberdous, M., Vandenbulcke, L., and Beckers, J.-M., 2009. Weekly satellite sea surface temperature around Corsica, a DINEOF analysis of AVHRR data (1998), foreseeing comparison with interpolated and modelled fields. ASLO aquatic sciences meeting 2009, Nice, France.
http://hdl.handle.net/2268/33595

Sirjacobs, D., Alvera, A., Barth, A., Lacroix, G., Park, Y., Nechad, B., Ruddick, K., and Beckers, J.M., 2011. Cloud filling of ocean color and sea surface temperature remote sensing products over the Southern North Sea by the Data Interpolating Empirical Orthogonal Functions methodology. Journal of Sea Research, 65(1): 114-130.
http://hdl.handle.net/2268/81349

Stackhouse, J., 1797. *Nereis britannica*; continens species omnes fucorum in insulis britannicis crescentium: descriptione latine et anglico, necnon iconibus ad vivum depictis. Bathoniae [Bath] & Londini [London]: S. Hazard; J. White., Fasc. 2. pp. ix-xxiv, 31-70, pls IX-XIII.

Süzgeç-Selçuk, S., Meriçli, A.H., Güven, K.C., Kaiser, M., Casey, R., Hingley-Wilson, S., Lalvani, A. and Tasdemir, D., 2011. Evaluation of Turkish seaweeds for antiprotozoal, antimycobacterial and cytotoxic activities. Phytotherapy research, 25(5): 778-83.
doi: 10.1002/ptr.3330

Tanner, C.E., 1981. Chlorophyta: life histories. Book Chapter in: Lobban, C.S. and Wynne, M.J., 1981. The biology of seaweeds. University of California Press, Botanical Monographs, 17, 786 pp.

Tempera, F., 2009. Benthic habitats of the extended Faial Island shelf and their relationship to geologic, oceanographic and infralittoral biologic features. UK, University of St. Andrews. PhD thesis, 341 pp.
http://www.geohab.org/docs/2009/Tempera.pdf

Tittley, I. and Neto, A.I., 1994. "Expedition Azores 1989": Benthic marine algae (seaweeds) recorded from Faial and Pico. Arquipélago. Ciências Biológicas e Marinhas, 12(A) :1-13.

Troupin, C., Lenartz, F., Sirjacobs, D., Alvera, A., Barth, A., Ouberdous, M. and Beckers, J.-M., 2009. Evolution of Western Mediterranean Sea Surface Temperature between 1985 and 2005: a complementary study *in situ*, satellite and modelling approaches. 6th EGU General Assembly, 2009, Vienna, Austria.
http://hdl.handle.net/2268/6675

Troupin, C., Machin, F., Ouberdous, M., Sirjacobs, D., Barth, A. and Beckers, J.-M., 2010. High-resolution climatology of the North-East Atlantic using Data-Interpolating Variational Analysis DIVA. Journal of Geophysical Research, 115, 20.
http://hdl.handle.net/2268/68400

Trowbridge, C.D., 1998. Ecology of the green macroalga *Codium fragile* (Suringar) Hariot: invasive and noninvasive subspecies. Oceanography and Marine Biology: an annual review, 36: 1-64.

Tyberghein L., Verbruggen H., Pauly K., Troupin C., Mineur F. and De Clerck O., 2011. Bio-ORACLE: a global environmental dataset for marine species distribution modeling Global Ecology and Biogeography: published online.
doi: 10.1111/j.1466-8238.2011.00656.x

Uki., N., 1984. Abalone culture in Japan. Proceedings of the Ninth and Tenth U.S.-Japan Meetings on Aquaculture, Carl J. Sindermann (Ed.), NOAA Technical Report NMFS, 16: 83-88.

Van den Hoek, C., 1982a. The distribution of benthic marine algae in the relation of the temperature regulation of their life histories – Biological Journal of the Limnean Society, 18(2): 81-144.

Van den Hoek, C., 1982b. Phytogeographic distribution groups of bentic marine algae in the Nord Atlantic Ocean. A review of experimental evidence from life history studies - Helgolander Meeresunters, 35: 153-214.

Van den Hoek, C., 1987. The possible significance of long-range dispersal for the biogeography of seaweeds - Helgolander Meeresunters, 41: 261-272.

Van den Hoek, C., Mann, D.G. and Jahns H.M., 1995. Algae. An introduction to phycology. Cambridge University Press, Cambridge, U.K., 623 pp.

Vaqué, D., Agusti, S., Duarte, C. M., Enriquez, S. and Geertz-Hansen, O., 1994. Microbial heterotrophs within *Codium bursa*: a naturally isolated microbial food web. Marine Ecology Progress Series, 109: 275-282.

Verbruggen, H., Leliaert, F., Maggs, C.A., Shimada, S., Schils, T., Provan, J., Booth, D., Murphy, S., De Clerck, O., Littler, D.S., Littler, M.M. and Coppejans, E. 2007. Species boundaries and phylogenetic relationships within the green algal genus *Codium* (Bryopsidales) based on plastid DNA sequences. Molecular Phylogenetics and Evolution, 44: 240-254.

Verbruggen, H., Ashworth, M., LoDuca, S. T., Vlaeminck, C., Cocquyt, E., Sauvage, T., Zechman, F. W., Littler, D. S., Littler, M. M., Leliaert, F. and De Clerck, O. 2009. A multi-locus time-calibrated phylogeny of the siphonous green algae. Molecular Phylogenetics and Evolution, 50: 642-653.

Vidondo, B. and Duarte, C.M., 1995. Seasonal growth of *Codium bursa*, a slow growing Mediterranean macroalga: *in situ* experimental evidence of nutrient limitation. Marine Ecology Progress Series, 123: 185-191.

Vidondo, B. and Duarte, C.M., 1998. Population structure, dynamics and production of the Mediterranean macroalga *Codium bursa* (*Chlorophyceae*). Journal of Phycology, 34(6): 918-924. doi: 10.1046/j.1529-8817.1998.340918.x

Vroom, S. and Smith, C.M., 2001. The challenge of *siphonous* green algae. American Scientist, 89(6): 525-531.

Weber-van Bosse, A., 1896. On a new genus of siphonean algae - *PseudoCodium*. Journal of the Linnean Society of London. Botany, 32: 209-212.

Williams, M.M., 1925. Cytology of the gametangia of *Codium tomentosum* (Stckh.). Proceedings of the Linnean Society of New South Wales, 50: 98-111.

4 - The potential of visible remote sensing for benthic ecological studies

Table of contents

4.1 Benthic ecology dynamics requires spatial considerations

In order to link realistically and at a large scale the marine habitat status with notions of sustainable development of marine coastal uses, new extensive surveying approaches are required in complement with the traditional approaches developed in localized benthic research. However, in the coastal environment, precise habitat description and population monitoring are particularly difficult to achieve. New emerging techniques are increasingly needed to allow more extensive, frequent, and precise monitoring. These techniques include processing of images (visible or acoustic) obtained from platform ranging from satellites in space down to underwater automated robots or handled in scuba-diving, as well as the permanent installation of seabed sensors. Still to be combined with classical field work monitoring, sampling and laboratory studies, this information can be integrated into environmental and spatialized modelling.

Extensive, precise and frequent seabed surveying constitutes an expensive monitoring work but is the price to pay to allow a rational management of resources without taking the risk of damaging them further. Yet, modern technology is progressively reducing these monitoring costs by expanding our abilities in term of imaging resolution, navigation automation and range, and subsequently in term of spatio-temporal coverage efficiency.

Modern seabed mapping becomes thus a basic requirement for the general study of marine ecosystem processes and changes, the implementation and long term monitoring of Marine Protected Areas (MPAs), the management of marine resources, and the monitoring and modelling of species' distributional shifts.

4.1.1 Habitat mapping and Marine spatial ecology

Following a review of the collective contribution that acoustic and optical imaging has made to benthic ecology, Solan *et al.* (2003) underlined that benthic acoustic or optical reconnaissance survey techniques are invaluable and a necessary first step to gain a habitat-scale knowledge and appreciation of the system one is about to study; as "getting a large-scale look at the forest is always a good idea before one starts using point-sampling techniques to look at individual trees, branches or leaves".
Landscape ecology, a top-down approach to understanding ecosystem dynamics, considers all landscapes as a heterogeneous mosaic of landscape units, or habitat patches, which are repeated at intervals over space (Kent *et al.*, 1997). In terrestrial landscape ecology, habitat patches are often defined on the basis of vegetation type and/or anthropogenic structures (Parry *et al.*, 2003). Although a similar approach has been applied in the marine environment to investigate spatial distribution of seagrass habitats (Robbins and Bell, 1994), marine landscapes are usually defined using physical characteristics rather than biological structures (Zajac, 1999).

In their recent review, Brown *et al.* (2011) conclude that the advent of spatial ecological studies founded on high-resolution environmental data sets will undoubtedly help to examine patterns in community and species distributions, providing thus improved spatial information for management of marine systems.

4.1.2 Examples of marine spatial ecology approaches

Some examples of application involving macroalgae spatial distribution can be found in the domain of environmental quality indicators. In the Bay of Calvi (Corsica, France), the spatial distribution, seasonal fluctuations and decadal trends of various macro algae are considered in regard to environmental factors and anthropogenic pressures (Demoulin *et al.*, 1980; Janssens *et al.*, 1993; Janssens, 2000). Modelling approaches of the interaction between seaweeds and eutrophication problems also requires spatialized considerations (Alvera-Azcárate *et al.*, 2003). Another example is the representation of the proportion of *Cystoseira amentacea stricta*, *Cystoseira compressa* and cumulated species of Ulvales along the coast of the Marine Protected Area of Portofino Promontory Park in the Ligurian Sea with the use of Geographic Information System (GIS) software (Mangialajo *et al.*, 2003).

Around the Faial and Pico islands (Azores, central group), a predictive study of the spatial distribution of numerous seaweeds (articulated *Corallinaceae*, *Codium elisabethae*, *Dictyota* spp., *Halopteris filicina*, *Padina pavonica* and *Zonaria tournefortii*) versus the major environmental variables characterizing their reef habitat (depth, slope, swell exposure, maximum tidal currents, SST and chl-*a* concentration) have allowed some refined understanding and new hypothesis concerning the ecological habitat requirements of these species (Tempera, 2009).

Some mapping of seagrass patches, as produced in the Cheasepeake bay (Orth *et al.*, 2008;) allows marine scientists to analyse the links between spatial distribution of habitats and the connectivity of fish populations (Jones, 2009). Such studies produce an estimation of the relative importance of each habitat regarding a specific population, highly valuable information for the design of protection measures.

In the same line of reflection, the fundamental studies that evaluate the links between ecosystem functions and biodiversity increasingly take into account the spatial notions of habitat fragmentation and subsequent population connectivity's (Matthiessen *et al.*, 2007) underlying again the importance of the preliminary establishment of population distribution maps.

More specifically, studies of the progression of invasive species (Schaffelke *et al.*, 2006; Verbruggen *et al.*, 2009a) are reaching a stage where spatial information on pre-existing habitats and populations becomes an unavoidable clue.

Worldwide, many political decisions were instructed for the design of networks of high biological values areas identified as key areas for good ecosystem functioning. According to situations, these areas are simply recognized as important zones, others benefit of a minimal monitoring effort, and finally some obtain variable status of protection with more or less efficient mechanisms of control (protection laws, executive surveillance and application of penalties). In this direction, the Californian state of the United States of America has made one example of very important investments with 1) the implementation of dense network of 101 Marine Protected Areas fully protecting marine life in 11 per cent of coastal waters in the region (Californian State Department of Fish and Games, 2010; Ocean Conservancy, 2011), and 2) the foundation of monitoring and controlling organisms interacting with all involved stakeholders (Moll *et al.*, 2009).

4.1.3 Marine spatial ecology at a starting point but prone to rapid evolution

Although these few examples, marine spatial ecology can still be considered a young science but technological development let foresee fast evolution (Kritzer and Sale, 2006). According to a literature review of Pauly and De Clerck (2010), marine spatial ecology is barely at a starting point if one considers that only 8 % of publications involving GIS, Remote Sensing and mapping are devoted to the coastal and marine domains. Further, within these 8 % of marine spatial studies, most publications concern supratidal communities (mainly mangroves) or non-biological issues (topography, geomorphology, nautism), while studies of benthic marine communities reach only 10 % for the most known coral and seagrass communities, and a maximum of 1% for macroalgae.

With the advent of molecular markers, the science of biodiversity conservation (conservation biogeography and genetics) can now be seen as linked with the broad traditional research fields of systematics, biogeography and population biology through several specific sub-disciplines (Fig. 1) amongst which various have an emerging spatialized analysis component (Diniz-Filho *et al.*, 2008, Provan *et al.*, 2008).

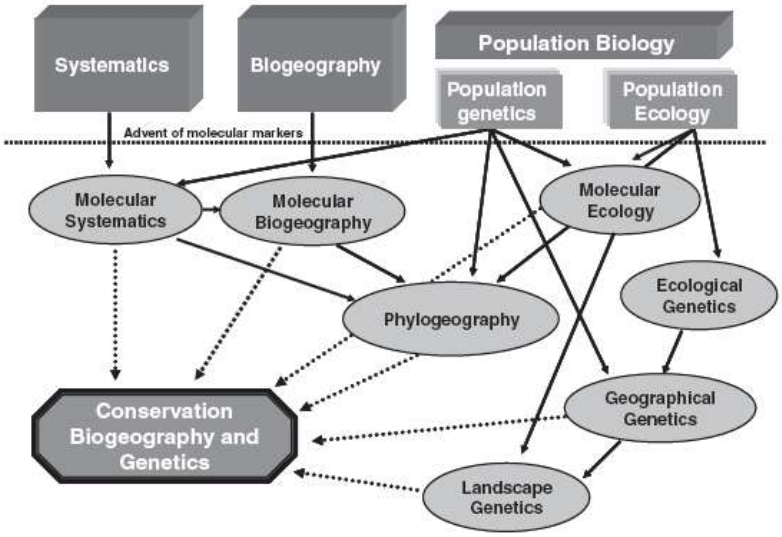


Figure 1- Schematic relationship among traditional research fields, specific sub-disciplines and their relationship with biodiversity conservation (conservation biogeography and genetics). Rectangles represent broad and traditional research fields, from which, after the development and popularization of the use of molecular markers, originated the research programs listed in the ellipses (extracted from Diniz-Filho *et al.*, 2008).

With the progress of genetics and spatial ecology, links between macroecology and macroevolution of organisms are appearing, as for the macroalga *Halimeda tuna* (Verbruggen *et al.*, 2009b). Recent European Commission report (European Commission, 2010) clearly concludes that action is now needed at EU level to ensure that Maritime Spatial Planning is deployed in the most coherent and effective way possible across sea basins to the benefit of both the development of maritime activities and the protection of the marine environment. This requires the integration of various approaches including habitat mapping, population distribution and dynamics, population genetics. **Ecological information's derived from seabed visible imagery will obviously play an important role in the construction of benthic metapopulation analysis, integrating space-borne and air-borne views of habitat connectivity, but also down to high resolution underwater imaging adapted to reveal precise changes in patchiness and dynamics.**

4.2 Synthetic history of benthic ecosystem mapping

This sections presents the general groups of benthic ecosystem mapping applications historically organized from the emergence of spatial ecology applications till today's concerns on underwater visible imagery mapping and key research points for the future. The biogeographical compilations of known stations will not be reviewed in this section, which focuses more on direct mapping approaches, focusing mainly on early experiences in the French coasts of Mediterranean. Little information on acoustic mapping is included hereafter in order to place the emergence of visible mapping within the global context. While reviewing the mapping approaches of seagrass and macroalgae, Dahdoud-Guebas *et al.* (1999) underlined the importance of the seagrass ecosystem and the evident need for mapping their distributions and identified Ascherson (1871) as having published the first seagrass distribution map.

4.2.1 Early mapping experiences in the French Mediterranean coast

Ollivier (1929), mapped of *Posidonia* and *Caulerpa* fields, but positioning precision was low and lower extension limits were not reached. Allenbach *et al.* (1973) used aerial photo with seagrass bed delineation, but without species confirmation were limited to –10 m. Kremer (1974) produced maps of seabed's nature of the French Mediterranean coast between Cape Martin and the Italian border. The presence of seagrass beds are reported but without species identification.

In 1972, Fredj produced one very early literature reference of underwater manned submarine use for exploration of benthic macroalgal community with a dive report on the *Laminaria rodriguezii* deep habitats at the vicinity of the Revellata (Corsica). The French Navy submarine "Griffon" was exploited in conjunction with acoustic positioning devices installed on the seabed to follow and map long extensions of the lowest limit of the *Posidonia oceanica* beds in the French Alpes Maritimes coastal area. Every minute and with coordinate clocks, both position of the submarine was recorded by surface teams while observer in the submarine recorded the seagrass coverage presence. The first series of dives were carried out in 1976 and allowed to follow 23 km of the inferior seagrass bed (Augier and Boudouresque, 1979; Meinesz and Laurent, 1978 and 1980). A second serie of dives carried out in 1977 were devoted to map another 10 km af *P. oceanica* bed inferior limit (Meinesz and Laurent, 1982), but with an enhanced discrimination of 3 beds types (plagiotropic growth, orthotropic growth, or thick 'matte') and of 2 health status according to earlier observations (Meinesz and Laurent, 1978). The absence of visible record and the approximate positioning precludes a change detection exercise.

In the first review of cartographic studies of seabed occupation by seagrasses, Meinesz and Lefèvre (1978) produced an estimation of all habitat destruction affecting the infralittoral in the French Alpes-Maritimes coast, based on studies produced from 1929 till 1974. Although the approach was limited to the horizontal surface projections (no consideration of seabed slope and morphology) of two sections (from 0-10 m and from 10-20 m depth), the authors could identify 10 ecological homogeneous sectors within the region. They mentioned that seagrass cartographic knowledge were often poor, incomplete and imprecise and raised the call for new extensive mapping efforts for any sound coastal planning and monitoring.

Some early studies of the spatial organization and dynamic of macroalgae can be illustrated by 3 types of applications achieved by Meinesz (1980). (1) A largest scale approach consisted in displaying the distribution of stations where particular algae have been identified in scuba diving as present, or as disappeared. Stations were pointed out by arrows on coastline maps at scales ranging

from the whole Western Mediterranean down to small bays extending over few kilometers. This scale corresponds to the scope of a large to medium scale static description of biogeographical distribution. (2) Over a more limited area of 20 meter long and few meters wide, the evolution, progression and dissemination of *Zoostera noltii* and *Caulerpa prolifera* was mapped to follow an experiment of implantation in a shallow (-2.5m) sandy-muddy environment. The cartography produced in scuba diving consisted in hand drawing schemes delineating the occupation of seabed and were repeated 5, 7 and 13 months after the implantation. (3) At very close scale finally, Meinesz (1980) made a quantitative description of the morphology development, growth and colonization modes of *Halimeda tuna* by repeating *in situ* drawings of silhouette and position of all thallus located with a 4 cm grid within a fixed quadrat of 20 cm by 20 cm. This time consuming scuba diving manual recording of small scale structures was carried out at a 5 meter depth station and repeated 6 times during a period of one year, allowing compiling quantitative information on the propagation, growth and development of the articles of *Halimeda tuna*.

In 1983a, Meinesz *et al.* published an inventory of marine protected areas of the French Mediterranean coasts, where they concluded by the urgent necessity for France to implement an efficient network of marine reserves in order to protect and rationally exploit it's coastal patrimony. Still in their synthesis of 1991a, Meinesz *et al.* continued to underline that over most of the coast studied, the lower limits of *P. oceanica* beds were not mapped precisely, even if some new mapping and monitoring approaches involving diving, side scan sonar and submarine excursion did started (; Falconetti and Meinesz, 1982; Meinesz *et al.*, 1981).

In the context of the optimization of the methodologies used by a growing number of authors Meinesz *et al.* (1983b) proposed a standardization of the symbols used in the representation of Mediterranean seabed maps and transect, with a series of 69 symbols proposed for main communities over both minerals or biogenics substrates types.

In situ scuba diving approaches were standardized for the mapping of submarine vegetation communities (Boudouresque *et al.* 1985; Meinesz and Simonian, 1983; Meinesz and Lefèvre, 1984). The methodology consisted in human scheme drawing of communities distribution observed along transects of seabed localized in space relatively to reference marks (buoys or land marks) with a net of moving graduated lines and decameters (isolated transects in Meinesz and Simonian, 1983; radial transects in Meinesz and Lefèvre, 1984; gridded transects in Boudouresque *et al.*, 1985).

Change detection mapping work by Meinesz and Lefèvre (1984) derived temporal regeneration dynamic of *Posidonia oceanica* meadows after the impact of a bomb, in a circular area of 85 m of radius ranging from 6 to 15 meter depth. They carried out a mapping of this area by SCUBA diving using 2 horizontal weighted measuring lines radiating from a central metallic "stick" fixed in the sediment, and delimiting a sector of the circle. Propagating a decameter along these reference lines on the occasion of 13 dives, they could locate and record the position, shape and size of every recolonizing *P. oceanica* tufts within the circle, with an announced position precision of 50 cm. From these data, they could produce a histogram of tufts diameters (from 0.5 m till 6.5 m wide; and subsequently evaluate their mean horizontal growth rate (3.75 cm/year) and implantation rate (3 vegetative recruits/ha/year), since the known destruction of the original meadow by the explosion. They observed earlier aerial photographs that globally confirmed their mapping interpretation concerning the overall distribution and degree of meadow habitat destruction, but mentioned that the quality of these images would only have allowed to distinguish *P. oceanica* recolonization spots larger than 2 meter wide, and this, for the shallowest part between 10 and 5 meter deep. This study is really interesting as it is a very nice illustration of the scales at which a precise mapping of size

and structure of benthic populations can indirectly answer to particular ecological questions, fundamental in a spatial ecological approach. It also illustrated one of the early interests to use airborne visible imagery to answer such questions, but also the need to achieve complementary work *in situ* both for reaching the requested resolution and to cover the deepest part of the zone under study.

Some 10 years later after the sole *in situ* diving mapping approaches were standardized (Boudouresque, *et al.* 1985; Meinesz and Simonian, 1983; Meinesz and Lefèvre, 1984), they were still considered as reference and used when relatively precise mapping are required over limited areas with depth ranging from 5 to 25 meters, as for instance on the occasion of 1 hectare mapping within the Port-Cros national park (Var, France) (Bellone and Meinesz, 1995; Loques *et al.* 1995). The positioning precision reached with *in situ* transect mapping supported with remote sensing was announced to range from 0.5 m (Meinez and Lefèvre, 1984) till 2 m (Boudouresque *et al.* 1985).

4.2.2 Early exploitation of airborne and satellite imagery

Boudouresque, *et al.* (1985) mapped very peculiar shallow reef formations of *Posidonia oceanica* beds present in the bay of Saint-Florent in Corsica, together with the other associated seagrasses and photophilous algae communities. They used a perpendicular gridded lines transect methods allowing divers to draw the observed repartition of communities within each sub sector of 10 by 10 m and produced this way a mapped coverage of 7.3 ha with a precision of localization estimated at 2 meters. They also exploited aerial photography to extend their map to an area of 32 ha, using the first map as ground truth, together with complementary dives.

Few years after, Meinesz *et al.* (1988) demonstrated a combined approach for the mapping of seabed communities in the clear waters of Corsica. As first step, they visually exploited a low altitude aerial photography to establish a first draft map of coast line and seabed occupation limits for which some distinctions could be made down to a depth of 15 m. In second step, they used this map in scuba diving to follow and control boundaries and patches, as to identify various new features that couldn't be identified by aerial photography. For instance *Posidonia oceanica* meadows, drift leaves accumulations and dead 'mattes' mosaics all appeared dark blue on the such aerial photography whereas both continuous dead 'mattes' and rocks bearing photophilous algae appeared brown. As final step, they explored few control transects in scuba diving along graduated ground lines fixed to the shore line at known positions. With an accuracy of 1-2 meters, this approach was presented as an efficient low cost monitoring method, more adapted to such shallow small scale coastal mapping than satellite remote sensing or side-scan sonar. If large long term changes in seabed occupation could be apprehended with such precision, it would not be sufficient to resolve the smaller seasonal or even annual spatial dynamics of the seabed ecosystem and seabed communities limits (ie. considering the low horizontal growth rates of *P. oceanica* 3.75 cm/year). The purely *in situ* survey of smaller localized permanent quadrat (Boudouresque *et al.* 1981, 1986) was yet considered by Meinesz *et al.* (1988) to be more suitable for high accuracy long term monitoring.

The potential of the Spot satellite was evaluated for benthic communities mapping in the Mediterranean, first with simulated data acquired with airborne radiometer over the bay of San Cyprianu (Corsica) (Belsher *et al.*, 1988), then with real Spot data north of the Porquerolles island (Meinez *et al.*, 1991b). The latter study showed that these Spot data (green band XS1, red band XS2 and infra-red band XS3 with 20 meters resolution) could only produce a distinction of very contrasted seabed types (as sand / dense seagrass beds or sand / dense populations over rocks), but only within the depth range of 0-10 m and for the limited regions where a low declivity expands

over relatively large area allowing the 20 by 20 meter pixels to fall within homogeneous biocoenoses. Important additional limitations are imposed by rough sea surface conditions or important water turbidity, intense epiphytism over seagrasses, and solar angle causing sun glint.

4.2.3 Early exploitation of underwater imagery

The early examples of documented seabed photo mosaics seen in the literature concerned wrecks, as in the case of the sunken submarine Thresher (Ballard, 1975).

During a study of the benthic bioceonosis distribution of the “Principauté de Monaco” made by remote controlled video system (Fredj, *et al.*, 1993), fixed *Caulerpa taxifolia* were reported down to 73 meters. Using towed video, Belsher *et al.* (1994) expanded that observation down to 87 meters. The use of the manned submarine mapping approach was again used to explore further the lower distribution limit of *Caulerpa taxifolia* off Cap Martin and off Monaco in France (Belsher and Meinesz, 1995). The human observation was this time helped on wider angle by two camera videos and with lighting. This study showed that this alga occurs principally down to 60 meters with some rare but well developed fixed thallus down to 99 meters, constituting thus the principal macroalgae present between 45 and 100 meters. This mapping exploration showed that the natural spread of this introduced species proceeds not only horizontally but also vertically.

In the end of the 1980’s and early 1990’s, (Carleton and Done 1995) examined the feasibility of reliably estimating per cent cover of coral reef benthos by video techniques and concluded that for broad taxonomic categories of coral reef benthos, reliable estimates of relative abundance could be obtained by video techniques, showing strengths in the cost-savings in field expenses, and in the production of a permanent visual record, while being limited to reduced taxonomic resolution when compared with “hands on” field techniques.

In 1998, Engel was among the first to document the change in biodiversity imposed by trawling. Using sonar, video images taken from submarines and samples of seafloor life, the author compared a repeatedly trawled seabed 180 kilometers off the central California coast with a nearby swathe that was dragged less often. As underlined by Malakoff (1998), the heavily trawled area was flatter and harbored fewer species than the lightly fished stretch. They also found that increased trawling reshuffled sea life communities: smaller, rapidly reproducing creatures--such as nematode worms--tended to replace larger, longer-lived organisms, such as some shellfish.

Integrated approaches using airborne remote sensing, towed video, side scan sonar and completed with more traditional scuba diving and grab sampling techniques were proven optimal at several occasions, as for the mapping of seagrass and main facies around Port-Cros Island (Belsher *et al.*, 2005)

4.2.4 Benthic biota mapping experience in Calvi Bay (Corsica)

Several authors have contributed to the study of the spatial distribution of benthic communities in the Calvi bay, with evolving objectives, scales and technical means. A first map delineating 14 habitats was established from grab-sampling (Vaissière *et al.*, 1972). This work focused particularly on deeper communities and the coastal rocky algal habitats were localized with very little precision. Bay (1978) established a first map of P*osidonia* and C*ymodocea* beds in the whole Calvi bay, by projecting *in situ* observations made during localized or coast radial dives with topographical information. This map neglected the presence of rocky habitats and associated the upper limit of

seagrass beds with the coastline, except for large sandy patches. Moreover, its spatial precision was probably affected by large imprecision of bathymetry information available by then. In order to follow the distribution and temporal evolution of macroalgal communities in the bay of Calvi (Corsica, France), Demoulin *et al.* (1980) established a standardized methodology for scuba diving-surveys and for spatial representation of the algal abundance observed. The survey consisted in visual sensus of macroalgae qualitative abundance over a closed squared transect constituted of 2 bands of 1 m wide by 15 m length, one in subsurface and the other at a maximal depth of 15 meters, linked by ascending and descending 1 m wide transects. These surveys were repeated at regular intervals including stations of interest identified along the coast of the Calvi bay. Janssens *et al.* (1993) described the evolution of macroalga distribution after 12 years using the same survey strategy, but with a visual estimation of algal coverage classified with the Braun-Blanquet scale of abundance – dominance producing estimate within 6 categories of substrate cover (negligible; < 5%; 5-25 %; 25-50%; 50-75%; >75%). These charts of abundance distribution along the coast were established for 33 macroalgae or groups of macroalgae and over four periods (September 1990, December 1990, March 1991 and June 1991). In these charts, the variation of abundance along the coast is also displayed by two categories of abundance per transect, representing the range encountered during the local transect. In another work, Janssens (1993) synthesized the mean vertical zonation of macroalgae population encountered in a short portion of coast from Stareso harbor till the Cormoran's Rock. Resolving the along shore changes of this vertical spatial organization was not targeted in this approach, which aimed more at characterizing the large traits of a population of coastal transects considered uniform concerning their environmental context (solar exposition, exposition to hydrodynamics and to human impacts).

Koudil (1993) exploited SPOT satellite imagery to map limits of *Posidonia* and *Cymodocea* beds and of sandy habitats in Calvi and Saint-Florent bays (Corsica). With a spatial resolution of 20 m, this methodology presented the advantage of producing an instantaneous view of the shallow upper limits of seagrass beds, but several problems showed that satellite imagery did not represent a stand-alone approach for small scale coastal mapping. No information could be derived beyond depths of 15 meters and all shallow rocky habitats were classified as sand. Further, for the *Cymodocea*, only a shallow distribution was revealed (at few meters depth) along the sandy coast of Calvi bay, while no large fields were detected along the upper limit of *Posidonia* beds, as revealed by the earlier map established in scuba diving by Bay (1978).

In another approach involving exploitation of 3 airborne ektachrome photographies collected over the western part of the Bay of Calvi in 1990 and 1996, Janssens *et al.* (2000) produced a classification mapping of the seabed and could delineate three seabed categories: sand, rock and seagrass bed. According to the author, this distinction was efficient until a maximum depth of about 20 meters. Complementary diving work was thus required to complete the localization of lower rock and seagrass limits.

Going beyond the notion of presence/absence, mapping of the spatial variations of density, biomass and biometric or physiological parameters of the seagrass beds of *Posidonia oceanica* were also achieved from an extensive compilation of scuba diving measurements and samplings over a 4 ha study site (Gobert, 2002; Gobert *et al.*, 2003).

4.2.5 Benthic biota mapping around Laing Island, Hansa bay (Papua New Guinea)

While investigating distribution of blue-green algae around Laing Island in Hansa bay (Papua New Guinea), Hoffman (1988) could produce a cartographic survey of blue-green algal vegetation over the shallow coral reef plateau surrounding the island during low tide, while only general vertical zonation could be derived for the infralittoral zone (0-40 m), as consequence of the limitations of scuba diving both in study time and positioning precision. In the same area, Bay and Demoulin (1989) have later mapped the distribution of 4 seagrasses present between 0 and 7 m deep along a 5km portion of coast. They used 11 scubadiving transects to record distribution limits as well as localized measures of density and standing crop. Although they underlined the approximate location of transects and sampling points, they could produce an estimate of the seagrass annual primary production and identify wave action as most probable factor shaping seagrass distribution in this bay.

4.2.6 Recent state of the art review

In a review of a wide range of seabed-mapping technologies regarding their effectiveness in discriminating benthic habitats at different spatial scales, Kenny *et al.* (2003) produced a table illustrating the Area of seafloor mapped (per unit of effort) versus resolution for different sensing and sampling systems (table 1).

Table 1 -Area of seafloor mapped (per unit of effort) versus resolution for different sensing, acoustic and sampling systems (extracted from Kenny *et al.*, 2003)

System	Area mapped ($km^2 h^{-1}$)	Resolution (horizontal)								Remarks
		1000 m	100 m	10 m	1 m	1/10 m	1/100 m	1/1000 m	<1/1000 m	
Remote sensing, satellite	>100		●	•						Restricted to operational coverage, mainly shallow seas
Remote sensing, aircraft	>10	•	•	●	•					Generally restricted to depths <6 m
"Chirp" SSS	10		•	•	●					High-energy broad bandwidth pulse sonar
MBES	5		•	•	●	•				Allows the use of backscatter data to characterize substrata
SSS	3.5		•	•	•	●	•			Swath width depends on frequency used
Synthetic aperture sonar	3.0					•	●	•		Optimal operation in range of 50–100 kHz
AGDS	1.5		•	•	●	•				Valid for normal (narrow) beam surface coverage only
High-resolution sub-bottom profiler	0.8	•	•	•	●	•				Narrow-beam sub-surface coverage
Video camera	0.2				•	●	•			Allows mega-epibenthos identification
Benthic grab/core sampling	0.003					•	●	•		Quantitative data on macro- and meio-fauna requires laboratory analysis
Sediment profile camera	<0.001						•	●		Sediment/water interface inspections
X-ray photography	<0.001						•	●	•	High-resolution geochemical and physical inspections (water content, density)

4.3 Overview of benthic biota optical remote sensing studies

Focusing specifically on seaweeds, Pauly and De Clerck (2010) showed how geographic techniques such as remote sensing and applications based on geographic information systems are the key to document changes in marine benthic macroalgal communities. Seaweed communities mapping is a complex issue as they are usually heterogeneous due to the morphology of rocky substrates, characterized by many small scale differences in exposure to light, temperature fluctuations, waves, grazers and nutrients, resulting in many microclimates and patchy assemblages at the scale of several meters to less than a meter (Pauly and De Clerck, 2010). Further, algal vegetation often presents a mosaic structure of several associations closely imbriqued (Coppejans, 1977; Molinier, 1960).

Traditionally, spatial information was recorded in various ways in macroalgal benthic ecology, according to the scale targeted and observation mode. A large to medium scale biogeographic distribution approach consisted in reporting qualitative visual census from discrete stations onto coastline maps with particular symbols. Such approach was used at scales ranging from the whole Western Mediterranean (Meinesz, 1980) down to small bays extending over few kilometers, as by Demoulin *et al.* (1980) at the scale the bay of Calvi (Corsica, France). At smaller scales, the continuous spatial distribution of algae was traduced in two dimensional projection by hand drawing (ie by Meinesz, 1980, to follow an implantation experiment) or as an average one-dimensional vertical zonation of macroalgae population (ie by Janssens, 1993, describing a short portion of coast south of STARESO harbor, Corsica, France). At very close scale finally, early quantitative description of the algal morphology development, growth and colonization modes could be achieved by repeated scuba diving *in situ* hand drawings of silhouette and position of thallus located within a fixed quadrate, as done for *Halimeda tuna* (Meinesz, 1980).

The majority of remote sensing benthic biota investigations are conducted using optical images, of which mainly satellite and aerial images. Satellite imagery is mostly used for macro-scale studies (e.g. world-wide or country-wide distribution) while aerial photographs are usually suitable for higher resolution investigations on regional scales (e.g. zonation). Underwater imagery approaches are also developing, and are more suitable for precise investigation on species distributions, biodiversity, as quantification of species biomass, cover rate with rare applications reaching dynamic analysis.

4.3.1 Above surface remote sensing

4.3.1.1 Satellite remote sensing

Satellite remote sensing constitutes an ideal monitoring approach for applications requiring largest area coverage, but the spatial resolution is obviously lower than for other remote sensing application carried out closer to seabed. If one advantage is the stability of the platform comparatively to airborne remote sensing, the earth atmospheric influence can be of important concern in resolving the seabed nature, in addition to the water column complex influence. Satellite data are produced daily to biweekly according to the number of satellite in constellation and to the nature of their orbital configuration. This long term regularity in coverage constitutes one advantage for the space-borne approach, allowing for change detection at global scale.

Several impressing seaweed studies from space exist, with a trend in increasing information being derived beyond simple presence and distributions. Numerous applications focused on coral reef ecosystems in general and some on their marine vegetation (Belsher *et al.*, 1990). Efforts are today frequently invested in the distinction of species composition of macroalgae communities and linking *in situ* measurements to derive biomasses estimates from satellite views. For instance, foreseeing the harvesting of two invasive brown algae *Trubinaria ornata* and *Sargassum mangravense* having strong biotechnological potential for the cosmetic industries, Andrefouët *et al.* (2004) estimated their occupation and biomass on Tahitian coral reefs with 4 meter resolution Ikonos satellite data. Image classification resulted in an overall habitat map accuracy of 70% and mean areal dry matter biomass of these algae were estimated to range from 0.133 ± 0.046 kg/m² to 0.193 ± 0.067 kg/m².

Dierssen *et al.* (2008) have also exploited satellite data (MODIS) in order to produce maps of distribution of shallow algal communities in the Bahamas, with an objective of separating dominant macroalgae within different patches, and producing biomass estimations. Pauly *et al.* (2010) explored the potential of the PROBA/CHRIS sensors offering both high spatial and spectral resolutions to map the dynamics of seaweed and coral cover in an area influenced by seasonal upwelling in the Arabian Sea. They announced a maximum likelihood supervised classification accuracy ranging from 64 to 71 %, and illustrated dense macroalgal stands covering nearly all coral communities during summer while regressing to bare rock or turf communities during winter, with coral becoming the predominant bottom type. Dierssen *et al.* (2010) linked of *in situ* biomass and primary production measurements with remote sensing change detection in order to produce a spatially integrated estimate of the contribution of macroalgae primary production to the coastal ecosystem functioning of the Bahamas bank (Fig. 2). They showed a significant relation ($r2 = 0.64$, $p < 0.01$) between logarithmic comparison of satellite-modelled net primary production from stations throughout the banks and field campaign estimates and concluded that moderate to dense seagrass meadows of *Thalassia testudinum* were the dominant primary producers and contributed over 80% of net primary production in the region.

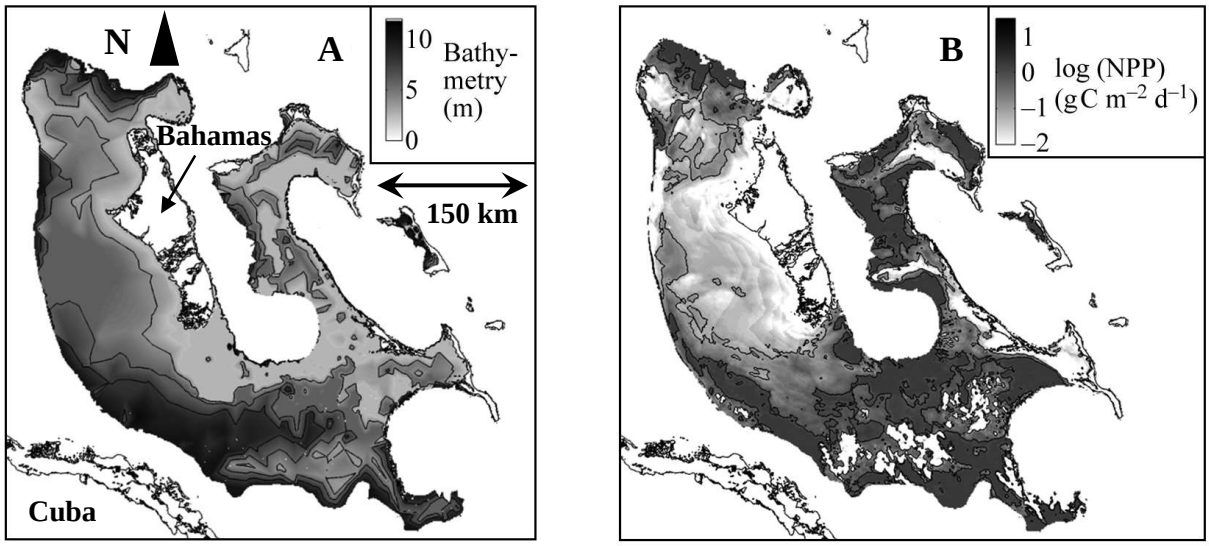


Figure 2- A) Digitized and gridded bathymetry across the Great Bahama Bank from 0 to 10 m; B) Modelled net primary production (gC m^{-2} yr^{-1}) derived from SeaWiFS imagery over the entire Bahamas Banks; adapted from Dierssen *et al.* (2010).

Those examples show the increasing potential of space borne remote sensing application to answer complex studies of ecosystem processes, along with the increasing spatial and spectral resolutions.

4.3.1.2 Airborne remote sensing

Airborne platforms have the advantage of allowing relatively easy and fast deployment of recent technologies and can thus provide very high spectral resolution, as spatial resolution in low altitude flight conditions. For instance, the Compact Airborne Spectrographic Imager (CASI) can resolve features measuring only 0.25m x 0.25m in up to 288 bands programmable between 400 µm and 1050 µm in the visible and near infrared light depending on the characteristics of the object studied (Pauly and De Clerck, 2010). These characteristics are increasingly used for exploring potential applications concerning coastal ocean color and water quality (Shimoni *et al.*, 2003; Sirjacobs *et al.*, 2003), as concerning benthic applications (Theriault *et al.* 2006). These last authors exploited the airborne hyperspectral sensor CASI (configuration of 19 bands of ocean color from 391-904 nm at 1m² spatial resolutions) to map benthic communities over 7 km² of Bahamas coast down to 6 m water depth. They interpreted a multiscale patchiness as indicative of on-going invasion dynamics and concluded that airborne hyperspectral technology is suitable for portraying the time-dependent outcomes of these dynamics at ecologically meaningful spatial scales. Gagnon *et al.* (2008) demonstrated the advantage of integrating digital bathymetry with hyperspectral data for the mapping of benthic assemblages in optically shallow waters, as it appeared to improve the accuracy of the classifications both by reducing the confusion among the spectral classes and by removing noise in the image data. High resolution acoustic mapping can in this case consist of preliminary information allowing exploiting optimally and to deeper limit the visible imagery.

4.3.2 Boat embarqued remote sensing

Visible mapping from direct boat physical support is scarcely developed and used as it is still affected by the important water column absorption and diffusion of visible light, without having the larger swath advantage of airborne systems. Usually, boats are rather used for towing underwater systems or divers going down to few meters above seabed, or as support ship for the deployment of ROVs (Robotized Oceanographic Vehicles) or AUVs (Autonomous Underwater Vehicles).

As described in Lirman (2007), the geospatial video-based survey technique SWaPS (Shallow-Water Positioning System, developed by scientists from NOAA's National Geodetic Survey) uses a GPS receiver attached to a video camera and can be operated from very light embarkations. The GPS receiver is centered over the digital video camera looking down at the bottom through a glass enclosure. During the surveys, each video frame recorded is stamped with complementary information as GPS position, but also date, depth, heading, pitch and roll. Thus the georeferenced digital frames obtained in these surveys can be easily analyzed to document patterns of abundance and distribution of submerged aquatic vegetation and other benthic organisms, as well as damage patterns, providing the depth and water visibility of the habitat under study allow image exploitability. The addition of a precise recording of acoustic topography of seabed could allow this system to provide precise scale to the visible image recorded by considering the 3D projections resulting from seabed topography.

4.3.3 Underwater optical remote sensing and imagery

Today, underwater visible imagery becomes one of the key issues in spatial investigation on benthic habitat and biological processes as it can offer numerous advantages: cost-efficient field survey coverage, high resolution permanent visible archive, non-destructive quantifications, change detection if precisely geocoded, reach of depths non-accessible to divers, and is less intrusive for benthic fauna than traditional observations.

For some accessible parameters, spatially continuous visible seabed mosaics allow integrating spatial heterogeneity at larger scale than for the same scuba diving time invested and avoid thus some of the bias linked with sub-sampling regarding the occupational heterogeneity of seabed habitats. After underlying the importance of habitat mapping, Greene (2007) stated that "there is a lack of an extensive biological information layer in the seabed spatialized database produced by the MESH project, for which visible imagery will bring new insight". Concurrently, several authors recently stressed the importance of gaining precise spatial vision on biological data for conservation reasons (Halpern *et al.*, 2008). Today, geocoded time series of high resolution mosaics becomes a crucial tool to resolve the dynamic of natural processes or climate-related researches in benthic marine biology (Ludvingsen *et al.*, 2007). Underwater visible mosaics time-series are a key for monitoring detailed benthic site evolution, becoming increasingly important for quantification in several domains: impact of fishing methods (Fosså *et al.*, 2002); degradation of stony corals climate markers (Sinclair *et al.*, 2006); patch dynamic of coral recruitment and competition with macroalgae over small-scale 1m² footprint (Mumby *et al.*, 2005); impact of fish aquaculture (Angel *et al.*, 2009; Jacquemart, 2009); ecological and archaeological investigation linked with offshore constructions (Ludvingsen *et al.*, 2007); detection and impact of legal or illegal dumping.

The developing seabed underwater imagery can be classified into three categories of applications according to the level of complexity of the information produced.

4.3.3.1 Illustration of species presence and exploration of habitats types

The first group of applications exploits the raw product imagery for simple illustration of species presence, qualitative evaluation of density, or preliminary exploration of large areas in search of particular habitats types, resources, or of traces of natural or human induced changes.

Some applications use imagery only in a first stage of research, as an efficient mean allowing first exploration of large areas with reduced or no scuba diving investment. This will allow the selection of specific study sites (scooter/video) where other types of *in situ* measurements and experimental set up will follow.

For instance, some seabed imagery applications are used to illustrate the impact of fish aquacultures cages on the benthic communities and particularly on the disappearance of benthic primary producers (Angel *et al.*, 2009; Jacquemart, 2009).

4.3.3.2 Density or distribution maps from human observation of geocoded images

A second group of applications aims at exploiting the imagery for identifying presence, repartition, and/or density of species within large areas. In these applications, more important work is devoted to the processing of recorded images, from post-processing analysis such as cover rate or densities estimations resulting from human observations of the images, association of geographic coordinate to each image, fusion of various images into general mosaics, till distribution limits of specific species. Some of these approaches might already allow recording of the state of a benthic ecosystem into an informatics mapping system and constitute good reference archive for the study of qualitative changes at large scale.

Concerning macroalgal abundance, Miller *et al.* (2003) could show the similarity between per cent cover measured from underwater video transects and of biomass estimated from harvested quadrates. They mentioned that numerous large-scale integrated coral reef monitoring programs doe use video transect sampling, but few of them do extract regularly macroalgal data. The Caribbean Coastal Marine Productivity (CARICOMP) program uses a line-intercept transect method for coral reef monitoring and reports calcareous, encrusting, fleshy, and turf algal categories (UNESCO, 1998). Although recognizing the need of technical improvements in their methodology, Álvaro *et al.* (2008) concluded that a point count human observation of seabed digital photography acquired with fixed frame could constitute a cost efficient alternative to *in situ* subtidal surveys for the definition of algal biotopes, when implemented during the whole monitoring.

The deployment of underwater cameras mounted on remotely operated vehicles (ROVs) permits comparable information to be collected beyond the depth and spatial extent achieved by SCUBA divers (Tempera, 2009), while providing non-destructive sampling with greater control of observations than possible with cameras mounted on towed vehicles (Parry *et al.*, 2003). However, the area of seabed observed in images collected by ROV is affected by variation of both camera height above the seabed and perspective associated with the camera angle relative to the seabed (inclination angle). Quantitative data has been extracted from ROV images for burrowing megafauna (Parry *et al.*, 2002 and 2003), but inter study comparison is possible only if vehicle velocity and camera orientation remain constant, as if the positioning is accurate enough, which is often the limiting factor requiring the help of manned image acquisition or fixed frame support for acceptable georeferenced sites.

Within this category of applications, some are progressing a step ahead by attempting to provide an exploitable scale associated with each image, through a simple measurement of the camera-seabed distance (depth of diver recorded by dive computer within image frame, difference between dive computer depth profile and towing boat echo-sounder records linked through time records into a GIS). The scale allows an estimation of organism’s size, yet it can remain quite imprecise as the seabed topography is a priori unknown at the scale of the image footprint, as the position and orientation (pitch, roll and yaw) of the camera objective at acquisition time.

4.3.3.3 Scaled underwater imagery as a precise measuring tool of organisms parameters

If several underwater imagery applications reached the stage of describing qualitative estimates seabed percentage cover of benthic biota species or groups, a little proportion of them aimed at applying geometric considerations for metric exploitation of the images. This third group of applications aims at exploiting imagery products as precise measuring tool, where position and scale can be attributed to every pixel, respecting some seabed topographical information and position of the camera relatively to its objective. Such goals can be achieved with simple technology involving mainly scuba diving work with commercial still and video cameras as demonstrated in the present work (see Chapter 7), with advanced mosaicking applied on high resolution cameras mounted on ROV (Lirman *et al.*, 2007; Fig. 3), or with complex industrial imaging devices translated within fixed frames over seabed producing high resolution bathymetry and 3D objects size detection from combined feature recognition and navigation data (Eustice *et al.*, 2005; Ludvingsen *et al.*, 2006 and 2007). Using high technology vision systems analysing the pattern of spots projected by a mutli laser array, the scales across an image and object 3D shape can even be deduced (Pilgrim *et al.*, 2000; Rhoads *et al.*, 2001).

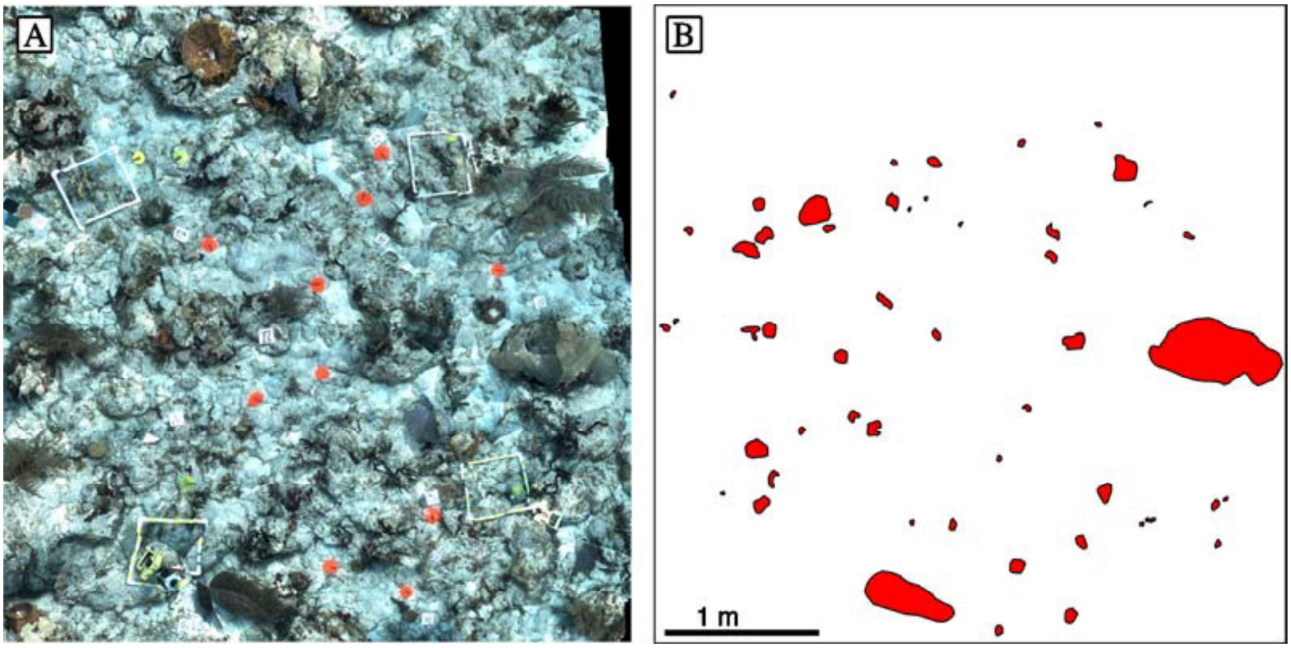


Figure 3- A) High-resolution video mosaic obtained with ROV; B) Abundance and spatial distribution of stony corals obtained by digitizing the boundaries of each coral colony. The coral cover obtained by this method (2.8%) was within the 95% confidence intervals of the values obtained by divers and from video mosaics using the point-count method (extracted from Lirman *et al.*, 2007).

In a study presenting the assessment of benthic resources and disturbance by Laser Line Scan Systems (LLSS) in turbid estuarine environments, Carey *et al.* (2003) mentioned that this technology is capable of imaging continuous swaths of the seafloor from 3 to 15 m wide at speeds up to 5 km/h. Image quality is dependent on system performance and turbidity but can exceed limits required to distinguish benthic habitats and census undisturbed fish and epibenthos. The areal coverage rate (1 km²/day) is announced by these authors to be several orders of magnitude higher

than video sleds and can complement high resolution acoustic surveys (multibeam or side scan). Holmes *et al.* (2008) demonstrated the possibility to make reliable estimates of the 3D surface area of entire colonies of complex branching coral species (*Pocilloporid*), using the plan view of the coral and a predetermined surface index function.

4.3.3.4 Benthic species growth rate and patch dynamics by change detection

Among the applications exploiting seabed images as calibrated measuring tools, only few are exploiting geocoded images time series to detect or quantify temporal changes of benthic populations. According the review by Mumby *et al.* (2005) concerning patch dynamic studies of macroalgae, while their cover is a core component of most reef monitoring programs, such studies rarely, if ever, investigate the dynamics of algae at the scale of individual patches.

Three studies were found to exploit time series of underwater visible imagery to document benthic organism evolution.

Mumby *et al.* (2005) used manual delineation (Fig. 4) of 1m² footprint images to estimate the relative proportion of coral/macroalgae and quantify the patch dynamics for two dominant species of macroalgae on a Caribbean forereef in Belize: *Lobophora variegata* (Lamouroux) and *Dictyota pulchella* (Hörnig and Schnetter). These authors insisted that the ecological functions of persistent algal patches are likely to be very different from those of highly dynamic patches, and that these kind of dynamics can optimally be addressed with underwater imagery.

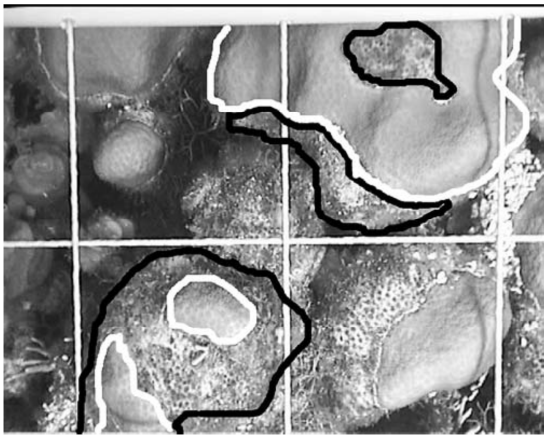


Figure 4- Upper surface of the massive coral *Montastreaea annularis* showing individual ramets and patches of live tissue. Parts of two ramets have been delineated using black for the edge of dead coral (colonized by algae) and white for live coral. (Each grid cell measures 10 by10 cm; extracted from Mumby *et al.*, 2005).

Focusing on Mediterranean coraligene and particularly sponges, Marquez I. Canals (2006) exploited long series (2002-2006) of small footprint images (below 1 meter) acquired with a fixed frame connected to the rock to describe lifespan and qualitative evolution of sponges.

Comparing 2 registered video mosaics acquired at one year interval by a ROV (Robotic Oceanic Vehicle), Lirman *et al.* (2007) mentioned the disappearance of 4 coral colonies out of 50 colonies over a 16 m² area but without quantifying organism's size or related change detection.
Many modern technologies are appearing in the field of seabed visible imagery, but with the increasing trend towards digital imaging, the immediate challenge lies not so much in developing new imaging tools, but developing more efficient ways for data analysis, storage, visualization and processing (Kenny *et al.*, 2003; Pauly and De Clerck, 2010; Solan *et al.*, 2003).

These last two categories of approaches are when there is a need to resolve the precise size and shape of species or archaeological objects, their relative positions (for patchiness, population structure or phytosociology in benthic ecology, history of ship wreck archaeology) or their temporal change detection (individual growth measurement, phenological stages succession or variation in population extension in benthic ecology, or layer recording during archaeological excavation).

4.4 Fast improving seabed visible imagery

4.4.1 Sensors

Focused specifically on above water visible remote sensing applications, the review by Pauly and De Clerck (2010) underlined the potential improvement of results to expect from the design of specifically oriented macroalgae sensors with high spectral resolution in the blue band, resolving better seabed signal through water column.

Facing the lack of high resolution hyperspectral imagery, pixel unmixing technics are developed to create synthetic data set exploiting the high spatial resolution of panchromatic bands to enhance the spatial resolution of the multispectral bands of the same sensor (Pauly and De Clerck, 2010). Such geostatistical techniques are used or planned for various applications, from enhancing spatial resolution of high spectral resolution imagery (i.e. for seabed coral and macroalgae mapping) to enhancing spatial resolution of high temporal resolution analysis of coastal ecosystem dynamics for the exploitation of geostationary sensors.

Another promising direction in the seabed mapping research field concerns the development of light-based active remote sensing, exploiting the detection of fluorescence induced in certain wavelengths following the emission of laser pulses at particular frequencies, exactly as known in optical microscopy, or airborne detection of phytoplankton groups. Laser line scanners are exploited since Funck *et al.* (1972), they are producing monochrome and less detailed information than classical optical cameras, but allow interesting fluorescence measurement of chlorophyll presence at a site (Jaffe *et al.*, 2001). Still, this technology is remaining complex to use, expensive and rare as few units have been produced. This technique was successfully exploited in laboratory conditions by Kieleck *et al.* (2001) to distinguish submerged green, brown and red seaweeds. Mazel *et al.* (2003) brought a multispectral fluorescence imaging system to underwater field conditions and managed to map various coral reef and macroalgae structures on a 1 cm resolution. Concerning this modern active remote sensing approach, Pauly and De Clerck (2010) supported the idea of its extension to airborne exploitation, foreseeing for instance a very high resolution mapping of the spreading of individual seaweed patches over coral reefs at relatively large scales. Although the increasing ground resolution of existing and proposed airborne remote sensing techniques, the

analysis of benthic macroalgae patchy dynamic might remain limited by the accuracy of the image georeferencing and by the water column absorption. For the analysis of small scale spatial dynamics processes as growth, patchy competition and colonization, the higher resolution underwater imagery approaches will probably remain prevailing compared to airborne.

4.4.2 Underwater acquisition

Future improvements are also to be expected in the field of underwater visible remote sensing from divers carried systems or fixed structure for small scale studies, or from autonomous underwater robots carrying high resolution low light sensitive cameras and lighting systems over larger scale applications.

High resolution imaging of the seafloor has been limited to fixed sensors or limited field-of view instruments. As a result, the ability to characterize large areas of benthic habitat has relied on acoustic maps and photomosaics combined with point sampling. Although underwater visible imagery constitutes an important step ahead for the monitoring of benthic environments, it nevertheless still faces some important challenges due to difficulties of underwater geolocalisation (Pauly and De Clerck, 2010), but also to correct projection and scaling in the mosaicking process, as well as to increasing possible extent of study area. For some parameters, underwater imaging acquired by human handled camera can be far more efficient in term of mapping coverage than for *in situ* visual census counting's but is still limited by scuba diving constraints and autonomy. Towed systems have also reduced field of view, but less autonomy problems. In the case of underwater robots exploitation for image acquisition, cost and energetic autonomy are the limiting factors.

Yet for the later automated approach, many modern developments in the field of Autonomous Underwater Vehicles (AUVs) are quickly expanding the potential of underwater remote sensing for biological applications, and their growing ability to navigate at controlled distance to seabed (Fig. 5) is allowing to foresee biological mapping at large scale and important depths (Singh *et al.*, 2004).

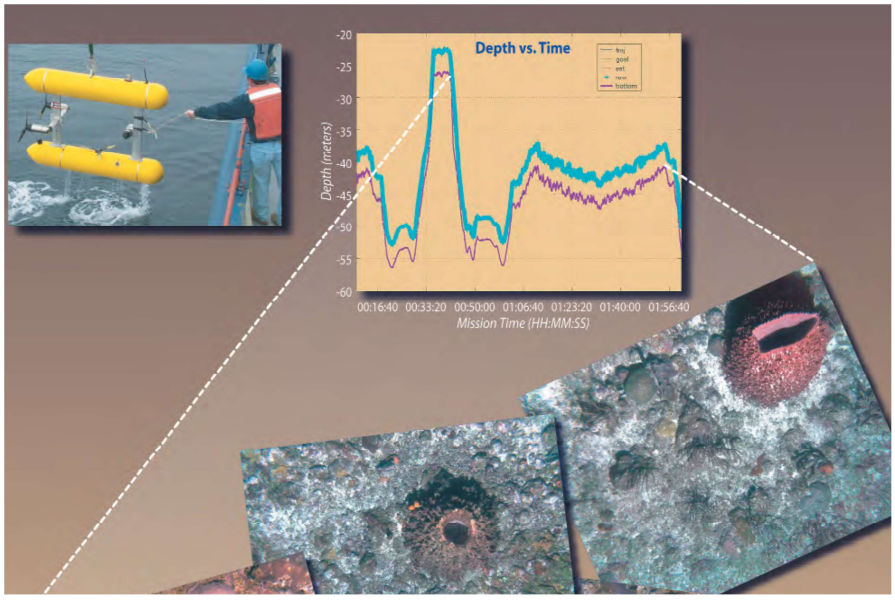


Figure 5- The Seabed AUV (top left) has been designed for high-resolution imaging close to the sea floor. As illustrated by the mission depth profile (blue line, top center) following bathymetry (purple line) at a height of 3 m height in varied terrain (coral reef in the U.S.Virgin Islands), it can obtain high-resolution high-quality color imagery (bottom row); (extracted from Singh *et al.*, 2004).

Important improvement of the potential of identification of seabed species and compounds is expected from AUVs by the use of spectral analysis of the seabed reflectance as for instance with multispectral cameras, but also in combination with spectral variations in the lightning allowing quantitative color analysis (Kirk, 1994), or even multispectral fluorescence detection (Jaffe *et al.*, 2001).

4.4.3 Image geocoding, mosaicking and scaling

Regular and large scale monitoring studies will become possible and will benefit from the developing modern navigation procedures (imagery based navigation, inertial navigation and magnetic field mapping, acoustic 3D geolocation), as from the greatly extending autonomy of AUVs.

To allow sound analysis of large amount of visible imagery, new analytical methods are required, and a first step prior to quantitative exploitation of this imagery is the mosaicking process.

Mosaicking process aims at wrapping a collection of overlapping images into a common coordinate system and merging the overlapped regions into a single image covering the entire scene. One part or the on-going developments are linked with the question of the mosaic constitution leading to the metric exploitability of images. The construction of high quality underwater mosaics of the sea bed is a whole research field under regular improvement (Gracias and Santos-Victor, 2000, 2001) producing increasingly robust results (Luong *et al.*, 2006) with developing technologies. With ROV applications for instance, Parry *et al.* (2003) exploited acoustic positioning and scaling of the recorded images (laser beams captured within the camera field of view and automatically processed). Lirman *et al.* (2007) exploited advanced and automated mosaicking schemes exploiting navigation information.

Since early nineties, image scale has been determined using structured lighting systems consisting of a pair of parallel laser diodes that project a pair of spots of known separation onto the seabed. Recent development of the Automated Benthic Image-Scaling System [(ABISS) (Pilgrim *et al.*, 2000)] allows calculation of scale across an entire image, accounting for variable camera height and perspective, by analyzing the pattern of spots projected by a 5-diode laser array.

4.4.4 Image analysis and classification

Algorithms are developed for specific applications, and most of them will attempt to automatize the image analysis as far as possible, reducing human intervention only where needed regarding the study objectives addressed and the robustness of alternate automatic treatment required. One of the main difficulty remaining to be solved is the robustness of such automatic algorithms regarding the diversity and temporal changes of seabed species development (epiphytes), of light and turbidity conditions, as of background reflectance (seasonal sand cover) (Sirjacobs, 2002; Marquez I. Canals, 2006). Variation of 3D perspective due to the camera position is also providing problematic interpretations in the segmentation process (Holmes *et al.*, 2008).

Yet approaches using simple and affordable commercial cameras can already achieve precise results for monitoring reference sites if a limited extension scale is targeted and is within reach of scuba-diving, providing good site preparation is made (see Chapter 7).

4.5 The present study and exploitation of seabed imagery

During MAROV project (see Chapter 1), the potential of underwater visible imagery was demonstrated for the study of presence and spatial distribution of benthic species around the Azores islands. Different approaches were exploited according to the ecological focus and scale, from ROV video transects along particular topographic features as tectonic ridges (Tempera, 2009) or shallow hydrothermal vents (Cardigos *et al.* 2005), to extended network of punctual video census spread around the island for biodiversity distribution study (Tempera, 2009).

Within this context, the present research exploited a 2 years' time-series of geometrically corrected seabed mosaics collected in scuba diving for the precise quantification of the *Codium elisabethae* macroalga populations dynamic within the SAC of Monte da Guia (Faial), as listed in the 2005 report of the Working Group on Habitat Mapping (WGHM), International Council for the Exploration of the Sea (Tempera and Santos, 2005).

4.6 References

Allenbach, M., Gloux, B., Morini, Y. and Sage, L., 1973. Etude de la plage Mala – Cap d'Ail (A.M.). Publication du laboratoire de Géologie et de Sédimentologie de l'Université de Nice, 9 pp.

Álvaro, N.V., Wallenstein, F.F.M.M., Neto, A.I., Nogueira, E.M., Ferreira, J., Santos, C.I. and Amaral, A.F., 2008. The use of digital photography for the definition of coastal biotopes in Azores. Hydrobiologia, 596:143–152.
doi: 10.1007/s10750-007-9064-7

Alvera-Azcárate, A., Ferreira, J.G. and Nunes, J.P., 2003. Modelling eutrophication in mesotidal and macrotidal estuaries. The role of intertidal seaweeds. Estuarine, Coastal and Shelf Science, 57: 715-724.

Andréfouët, S., Zubia, M. and Payri, C., 2004. Mapping and biomass estimation of the invasive *Turbinaria ornata* (Turner) J. Agardh and *Sargassum* (Grunow) Setchell on heterogeneous Tahitian coral using 4-meter resolution IKONOS satellite data. Coral Reefs, 23: 26-38.
doi: 10.1007/s00338-003-0367-5

Angel, D.L., Lefebvre, S., Holmer, M., García, T. and Pérez, M., 2009. New and innovative methods for monitoring ecological changes associated with aquaculture. ASLO Aquatic Sciences Meeting 2009, Nice, France, 25-30 January 2009.

Ascherson, P., 1871. Die geographische Verbreitung der Seegräser. Petermans Geographische Mittheilungen, 17: 241-248.

Augier, H. and Boudouresque, C.F., 1979. Premières observations dur l'herbier de Posidonies et le Détritique côtier de l'île du Levant (Méditerranée, France), à l'aide du sous-marin Griffon de la Marine Nationale. Travaux Scientifiques du Parc national de Port-Cros, 5: 141-153.

Ballard, R.D., 1975. Photography from a submersible during project FAMOUS. Oceanus, 18(3): 40-43.

Bay, D., 1978. Etude "*in situ*" de la production primaire d'un herbier de Posidonies (*Posidonia oceanica* (L.) Delile) de la baie de Calvi – Corse. Thèse de Doctorat en Sciences Océanologiques de l'Université de Liège, 251 pp.

Bay, D. and Demoulin, V., 1989. The seagrass beds of Hansa bay (north coast of Papua New Guinea). Bulletin de la Société Royale de Botanique de Belgique, 122: 3-17.

Bellone, E. and Meinesz, A., 1995. Cartographie sous-marine du Parc National de Port-Cros (Var, France). I – Elément de cartographie de l'herbier de *Posidonia oceanica* de la baie de Port-Man. Scientific report of the Port-Cros national park, Fr., 16: 123-128.

Belsher, T., Meinesz, A., Lefèvre, J.R. and Boudouresque, C.F., 1988. Simulation of SPOT Satellite Imagery for Charting Shallow-Water Benthic Communities in the Mediterranean. Marine Ecology, 9 (2): 157-165.

Belsher, T., A. Meinesz, C. Payri, and H. Ben Moussa. 1990. Apport du satellite SPOT à la connaissance des écosystèmes récifaux coralliens. La végétation marine de l'île de Moorea, Polynésie Française. Oceanologica Acta, 13(4): 513-24.

Belsher, T., Youenou, G., Dimeet, J., Raillard, J.-M., Bertrand, S. and Mereau, N., 1994. Elements cartographiques et évolution de *Caulerpa taxifolia* en Méditerranée (Alpes-Maritimes et Principauté de Monaco 1992). Océanologia Acta, 17(4): 443-451.

Belsher, T. and Meinesz, A., 1995. Deep-water dispersal of the tropical alga *Caulerpa taxifolia* introduced in the Mediterranean. Aquatic Botany, 51: 163-169.

Belsher, T., Houlgatte, E. and Boudouresque C.F., 2005. Cartographie de la prairie à *Posidonia oceanica* et des principaux faciès sédimentaires marins du Parc national de Port-Cros (Var, France, Méditerranée). Scientific report of the Port-Cros national park, France, 21: 19-28.

Boudouresque, C.F., Meinesz, A. and Panayotidis, P., 1981. Mise en place d'un carré permanent dans un herbier de Posidonies. Rapp. P. V. Réunion de la Commission Internationale pour l'Exploration Scientifique de la Méditerranée, 27 (9): 245-246.

Boudouresque C.F., Meinesz, A. and Lefèvre, J.R., 1985. Cartographie des peuplements benthiques marins de Corse. La formation récifale à *Posidonia oceanica* de Saint-Florent. Annales de l'Institut océanographique, 61(1): 27-38.

Boudouresque, C.F., Lefèvre, J.R. and Meinesz, A., 1986. Cartographie du carré permanent de la marina d'Elbu. Travaux Scientifiques du Parc Naturel Régional des Réserves naturelles de Corse, 2: 24-33.

Brown, C.J., Smith, S.J., Lawton, P. and Anderson, J.T., 2011. Benthic habitat mapping: a review of progress towards improved understanding of the spatial ecology of the seafloor using acoustic techniques. Estuarine, Coastal and Shelf Science, 92 (3): 502-520.
doi: 10.1016/j.ecss.2011.02.007.

Californian Department of Fish and Games, 2010. "Marine Life Protection Act Initiative" web page: http://www.dfg.ca.gov/mlpa/index.asp, accessed, 15/10/10.

Cardigos, F., Colaço, A., Dando, P.R., Ávila, S.P., Sarradin, P.-M., Tempera, F., Conceição, P., Pascoal, A. and Santos, R.S., 2005. Shallow water hydrothermal vent field fluids and communities of the D. João de Castro Seamount (Azores). Chemical Geology, 224: 153-168.

Carey, A.D., Rhoads, D.C. and Hecker, B., 2003. Use of laser line scan for assessment of response of benthic habitats and demersal fish to seafloor disturbance. Journal of Experimental Marine Biology and Ecology, 285-286: 435-452.

Carleton, J.H. and Done, T.J., 1995. Quantitative video sampling of coral reef benthos: large-scale application. Coral Reefs, 14(1): 35-46.

Coppejans, E., 1977. Bijdrage tot de studie van de wierpopulaties (Chlorophyceae, Phaeophyceae, Rhodophyceae) van het fotofiel infralittoraal in het noordwestelijk mediteraan beek en I, II et III. Doct. Sci. nat. Univ. Gent, 339 pp.

Dahdoud-Guebas, F., Coppejans, E. and Van Speybroeck, D., 1999. Remote sensing and zonation of seagrasses and algae along the Kenyan coast. Hydrobiologia, 400: 63-73.

Demoulin, V., Janssen, M. P. and Licot, M., 1980. Mise au point d'une méthode de cartographie des macroalgues marines : application à la région de Calvi (Corse). Lejeunia, 102: 1-68.

Dierssen, H.M., Burdige, D., Drake, L.A. and Zimmerman, R.C., 2008. Net primary productivity on the Bahama Banks revisited: seagrass, macroalgae, and grapestone. Proceedings of the Ocean Optics XIX conference, Braga, Italy, October 2008.

Dierssen, H.M., Zimmerman, R.C., Drake, L.A. and Burdige, D., 2010. Benthic ecology from space: optics and net primary production in seagrass and benthic algae across the Great Bahama Bank. Marine Ecology Progress Series, 411: 1-15.
doi:10.3354/meps08665

Diniz-Filho, J.A.F., Pires de Campos Telles, M., Bonatto, S.L., Eizirik, E., Renato de Freitas, T., de Marco Jr. P., Santos, F.R., Sole-Cava, A. and Nascimento Soares, T., 2008. Mapping the evolutionary twilight zone: molecular markers, populations and geography. Journal of Biogeography, 35: 753–763.

Engel, J. and Kvitek, R., 1998. Effects of otter trawling on a benthic community in Monterey Bay National Marine Sanctuary. Conservation Biology, 12(6): 1204–1214.

European Commission, 2010. Maritime spatial planning in the EU – Achievements and future development. Communication from the commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of the Regions. Brussels, 17 December 2010 - COM(2010) 771, 11 pp.
http://ec.europa.eu/maritimeaffairs/pdf/com_2010_771_en.pdf ; accessed 12/01/2011.

Eustice, R. 2005. Large-area visually augmented navigation for autonomous underwater vehicles. PhD thesis at the Massachusetts Institute of Technology and the Woods Hole Oceanographic Institution, USA, 187 pp.

Falconetti, C. and Meinesz, A., 1982. Charting the seaward limit of *Posidonia oceanica* meadows and of circalittoral biocoenoses along the coast of Monaco. Oceanologica acta, 12(4): 443-447.

Fosså, J.H., Mortensen, P.B. and Furevik, D.M., 2002. The deep-water coral *Lophelia pertusa* in Norwegian waters: distribution and fishery impacts. Hydrobiologia, 471: 1-12.

Fredj, G., 1972. Compte rendu de plongée en S.P. 300 sur les fonds à *Laminaria rodriguezii* Bornet de la Pointe de la Revellata (Corse). Bulletin de l'institut océanographique de Monaco, 42 pp.

Fredj, G., di Geronimo, S. and Gay, G., 1993. Inventaire et cartographie des biocénoses benthiques de la Principauté de Monaco. Département des travaux publiques et des affaires sociales, service de l'environnement, Principauté de Monaco, 40 pp.

Funk, C.J., Bryant, S.B. and Heckman, P.J., 1972. Handbook of underwater imaging system design (TP-303). Ocean technology department. Naval Undersea Center, 92 pp.

Gagnon, P., Scheibling, R.E., Jones, W. and Tully, D., 2008. The role of digital bathymetry in mapping shallow marine vegetation from hyperspectral image data. International Journal of Remote Sensing, 29 (3): 879-904.

Gobert, S., 2002. Variations spatiale et temporelle de l'herbier à *Posidonia oceanica* (L.) Delile (Baie de La Revellata-Calvi-Corse). Dissertation présentée en vue de l'obtention du grade de Docteur en Océanologie, Université de Liège, 2002, 207 pp.

Gobert, S., Kyramarios, M., Lepoint, G., Pergent-Martini, C., Bouquegneau, J.-M., 2003. Variations at different spatial scales of *Posidonia oceanica* (L.) Delile beds; effects on the physico-chemical parameters of the sediment. Oceanologica Acta, 26: 199–207.

Gracias, N. and Santos-Victor, J., 2000. Underwater video mosaics as visual navigation maps. Computer Vision and Image Understanding, 79: 66–91.
doi:10.1006/cviu.2000.0848

Gracias, N. and Santos-Victor, J., 2001. Trajectory reconstruction with uncertainty estimation using mosaic registration. Robotics and Autonomous Systems, 35: 163–177.

Greene, G., 2007. The importance of habitats mapping. MESH final Conference: "Mapping European Seabed Habitats, a framework to support sustainable management", 14-15/3/2007, Dublin Castle, Dublin, Ireland. http://www.searchmesh.net/pdf/Gary_Greene.pdf

Halpern, B.S., Walbridge, S., Selkoe, K.A., Kappel, C.V., Micheli, F., D'agrosa, C., Bruno, J.F., Casey, K.S., Ebert, C., Fox, H.E., Fujita, R., Heinemann, D., Lenihan, H.S., Madin, E.M.P., Perry, M.T., Selig, E.R., Spalding, M., Steneck, R. and Watson, R., 2008. A global map of human impact on marine ecosystems. Science, 319: 948-952.
doi:10.1126/science.1149345

Hoffman, L., 1988. Macroscopic benthic blue-green algal vegetation of Laing Island (northern coast of Papua New Guinea). Bulletin de la Société Royale Botanique de Belgique, 3: 3-11.

Holmes, G., Ortiz, J., Kaniewska, P. and Johnstone, R., 2008. Using three-dimensional surface area to compare the growth of two Pocilloporid coral species. Marine Biology 155: 421–427

Jacquemart, J., 2009. Etude de la communauté des macroalgues épiphytes de *Posidonia oceanica* (L.) Delile et comparaison avec celle de *Cystoseira balearica* Sauv. − Approche expérimentale visant à élucider les effets de l'eutrophisation sur ces communautés. Thèse de doctorat, Université de Liège, 393 pp.

Jaffe, J., Moore, K.D., McLean, J. and Strand, M.P., 2001. Underwater optical imaging: status and prospects. Oceanography, 14 (3): 66-76.

Janssens, M., 1993. Etat des peuplements algologiques de la baie de la Revellata (Calvi-Corse) : évolution, cartographie et biomasse. Mémoire de Maîtrise en Océanologie. Université de Liège, 71 pp.

Janssens, M., 2000. Etude *in situ* de la production primaire des macroalgues d'une baie méditerranéenne et influences dans le cycle du carbone : Baie de la Revellata, Calvi (Corse - France). Thèse de Doctorat en Sciences, orientation botanique, Université de Liège, 270 pp.

Janssens, M., Hoffmann, L. and Demoulin, V., 1993. Cartographie des macroalgues dans la région de Calvi (Corse) : comparaison après 12 ans (1978-79, 1990-91). Lejeunia, 141, 62 pp.

Jones, C.M., 2009. Can we use natural tags to measure connectivity and survival of fish? ASLO Aquatic Sciences Meeting 2009, Nice, France, 25-30 January 2009

Kenny, J., Cato, I., Desprez, M., Fader, G., Schüttenhelm, R.T.E., and Side, J., 2003. An overview of seabed-mapping technologies in the context of marine habitat classification. ICES Journal of Marine Science, 60: 411–418.
doi:10.1016/S1054–3139(03)00006-7.

Kent, M., Gill, W.J., Weaver, R.E., and Armitage, R.P., 1997. Landscape and plant community boundaries in biogeography. Progress in physical geography, 21: 315– 353.

Kieleck, C., Bousquet, B., Le Brun, G., Cariou, J. and Lotrian, J., 2001. Laser induced fluorescence imaging: application to groups of macroalgae identification. Journal of Physics D: Applied Physics, 34: 2561-2571.

Kirk, J.T.O., 1994. Light and Photosynthesis in Aquatic Ecosystems, 2nd ed. Cambridge University Press, Cambridge, 509 pp.

Koudil, M., 1993. Cartographie biosédimentaire des petits fonds marins par imagerie SPOT en Méditerranée. Golfes de Calvi et de St Florent. Mémoire de DESS Télédétection. Ecole Nationale des Sciences Géographiques, Université de Paris VI, 20 pp.

Kremer, Y., 1974. Littoral et précontinent de Menton (A.-M.). Morphologie, sedimentologie et structure. Thèse de doctorat de spécialité. Université de Nice, France. Laboratoire de Géologie et de Sédimentologie, 160 pp.

Kritzer, J.P. and Sale, P.F., 2006. Marine metapopulations. Ed. Kritzer and Sale, Elsevier Academic Press, Oxford, UK. 544 pp.

Lirman, D., Gracias, N., Gintert, B., Gleason, A., Reid, R., Negahdaripour, S. and Kramer, P., 2007. Development and application of a video-mosaic survey technology to document the status of coral reef communities. Environmental Monitoring and Assessment, 125: 59–73
doi: 10.1007/s10661-006-9239-0

Loques, F., Bellone, E., Meinesz, A. and Villette, M., 1995. Cartographie sous-marine du Parc National de Port-Cros (Var, France). II – La zone protégée de la Baie de la Palud. Scientific report of the Port-Cros national park, Fr., 16: 129-133.

Ludvingsen, M., Eustice, R. and Singh, H., 2006. Photogrammetric models for marine archaeology. In: Proceedings of IEEE/MTS OCEANS 2006, Boston, MA, September 18-21, 2006, pp.1-6.

Ludvingsen, M., Sortland, B., Johnsen, G. and Singh, H., 2007. Applications of geo-referenced underwater photo mosaics in marine biology and archaeology. Oceanography, Special Issue on Ocean Exploration, 20 (4): 140-149.

Luong, H.Q., Philips, P. and Foubert, A., 2006. Robust video mosaicing for benthic habitat mapping. In: Ranchordas, Alpesh, Araújo, Helder and Encarnação, Bruno (eds.) VISAPP 2006 - Proceedings of the First International Conference on Computer Vision Theory and Applications - 2 Volumes 2006, Setúbal, Portugal. pp. 306-310.

Malakoff, D., 1998. Papers Posit Grave Impact of Trawling. Science, 282 (5397): 2168.
doi: 10.1126/science.282.5397.2168b

Mangialajo, L., Cattanea-Vietti, R., Chiantore, M., Meinesz, A., de Vaugelas, J., and Barberis, G., 2003. Superficial algae as environmental quality indicators: application of a G.I.S. to the Portofino Promontory Park. Biologia Marina Mediterranea, 10 (2): 574-576.

Marquez I. Canals, E., 2006. Analyse de séries photographiques à long terme: étude de traits de vie d'espèces longévives des communautés du coralligène de Méditerranée. Master's Thesis. Université de la Méditerranée (Aix-Marseille II) Centre D'océanologie de Marseille.

Matthiessen, B., Gamfleldt, L., Jonsson, P.R., and Hillebrand, H., 2007. Effects of grazer richness and composition on algal biomass in a closed and open marine system. Ecology, 88(1): 178–187.

Mazel, C.H., Strand, M.P., Lesser, M.P., Crosby, M.P., Coles, B. and Nevis, A.J., 2003. High-resolution determination of coral reef bottom cover from multispectral fluorescence laser line scan imagery. Limnology and Oceanography 481 (1, part 2): 522-534.

Meinesz, A., 1980. Contribution à l'étude des *Caulerpales* (Chlorophytes). Avec une mention particulière aux espèces de la Méditerranée occidentale. Thèse de Doctorat, Université de Nice, France, 262 p.

Meinesz, A. and Laurent, R., 1978. Cartographie et état de la limite inférieure de *Posidonia oceanica* dans les Alpes Maritimes (France). Campagne Poséidon 1976. Botanica Marina, 21: 513-526.

Meinesz, A. and Lefèvre, J.-R., 1978. Destruction de l'étage infralittoral des Alpes-Maritimes. Bulletin d'Ecologie, 9(3): 259-276.

Meinesz, A. and Laurent, R., 1980. Cartes de la limite inférieure de l'herbier de *Posidonia oceanica* dans les Alpes Maritimes (France). Campagne Poséidon 1976. Annales de l'Institut Océanographique, Paris, 56 (1): 45-56.

Meinesz, A., Cuvelier, M. and Laurent, R., 1981. Méthodes récentes de cartographie et de surveillance des herbiers de phanérogames marines, leurs applications sur les côtes françaises de la Méditerranée. Vie et Milieu, 31(1): 27-34.

Meinesz, A. and Laurent, R., 1982. Cartes de la végétation sous-marine des Alpes Maritimes (côtes Françaises de la Méditerranée). 1 – Limite inférieure de l'herbier de *Posidonia oceanica* dans le Golfe Juan et à l'Est des îles de Lérins. Annales de l'Institut Océanographique, Paris, 58 (1): 103-112.

Meinesz, A. and Simonian, M., 1983. Cartes de la végétation sous-marine des Alpes Maritimes (côtes Françaises de la Méditerranée). 2 – La végétation mixte à Cymodocea nodosa – Zostera noltii – Caulerpa prolifera et la limite supérieure de l'herbier de *Posidonia oceanica* entre Juan-les-Pins et Golfe Juan. Annales de l'Institut Océanographique, Paris, 59 (1): 21-35.

Meinesz, A., Lefèvre, J.-R., Beurier, J.-P., Boudouresque, C.F., Miniconi, R. and O'Neill, J., 1983a. Les zones marines protégées des côtes Françaises de Méditerranée. Bulletin d'Ecologie, 14(1): 35-50.

Meinesz, A., Boudouresque, C.F., Falconetti, C., Astier, J.M., Bay, D., Blanc, J.J., Bourcier, M., Cinelli, F., Cirik, S., Cristiani, G., Di Geronimo, I., Giaccone, G., Harmelin, J.G., Laubier, L., Lovric, A.Z., Molinier, R., Soyer, J. and Vamvakas, C., 1983b. Normalisation des symboles pour la représentation et la cartographie des biocénoses benthiques littorales de Méditerranée. Annales de l'Institut Océanographique., Paris, 59 (2): 155-172.

Meinesz, A. and Lefèvre, J.-R., 1984. Régénération d'un herbier de *Posidonia oceanica* quarante années après sa destruction par une bombe dans la rade de Villefranche (Alpes-Maritimes – France). International Workshop *Posidonia oceanica* Beds, Boudouresque C.F., Jeudy de Grissac, A. and Olivier, J. edit., GIS Posidonie publications, France, 1: 39-44.

Meinesz, A., Boudouresque, C.F., and Lefèvre , J.-R., 1988. A map of the *Posidonia oceanica* beds of Marina d'Elbu (Corsica, Mediterranean). Marine Ecology, 9(3): 243-252.

Meinesz, A., Lefèvre, J.R. and Astier, J.M., 1991a. Impact of coastal development on the infralittoral zone along the southeastern Mediterranean shore of continental France. Marine Pollution Bulletin, 23 : 343-347.

Meinesz, A., Belsher, T., Boudouresque, C.F. and Lefèvre, J.R., 1991b. Première évaluation des potentialités du satellite Spot pour la cartographie des peuplements benthiques superficiels de Méditerranée occidentale. Oceanologica Acta, 14 (3): 299-307.

Miller, M.W., Aronson, R.B., and Murdoch, T.J.T., 2003. Monitoring coral reef macroalgae: different pictures from different methods. Bulletin of Marine Science, 72 (1): 199-206.

Molinier, R., 1960. Etude des biocénoses marines du Cap Corse. Vegetatio, 9(3-5): 121-312.

Moll, R.A., Mace, A., and Baird, B, 2009. California model for management of coastal ocean resources. ASLO Aquatic Sciences Meeting 2009, Nice, France, 25-30 January 2009. http://www.sgmeet.com/aslo/nice2009/viewabstract2.asp?AbstractID=5049

Mumby, P.J., Foster, N. L. and Glynn Fahy, E.A., 2005. Patch dynamics of coral reef macroalgae under chronic and acute disturbance. Coral Reefs, 24: 681–692. doi: 10.1007/s00338-005-0058-5

Ocean Conservancy, 2011. "California Marine Protected Areas" web page: http://www.oceanconservancy.org/site/PageServer?pagename=program_mpa , accessed 10/01/11.

Ollivier, G., 1929. Etude de la flore marine de la Côte d'Azur. Annales de l'Institut Océanographique, 7 (3): 173.

Orth, R.J., Wilcox, D.J., Kenne, A., Nagey, L.S., Owens, A. and Whiting, J.R., 2008. Distribution of submerged aquatic vegetation in the Chesapeake Bay and tributaries and Chincoteague Bay - 2007. Final Report U.S.E.P.A. http://web.vims.edu/bio/sav/sav07/?svr=www

Parry, D.M., Nickell, L.A., Kendall, M.A., Burrows, M.T., Pilgrim, D.A., Jones, M.B., 2002. Comparison of abundance and distribution of burrowing megafauna from diver and remotely operated vehicle observations. Marine Ecology Progress Series, 244: 89-93.

Parry, D.M., Kendall, M.A., Pilgrim, D.A. and Jones, M.B., 2003. Identification of patch structure within marine benthic landscapes using a remotely operated vehicle. Journal of Experimental Marine Biology and Ecology, 285-286: 497-511

Pauly K. and De Clerck O., 2010. GIS-based environmental analysis, remote sensing and niche modeling of seaweed communities, In: Israel A., Einav R. & Seckbach J. (eds.) Seaweeds and their Role in Globally Changing Environments, Springer, 480 pp. doi: 10.1007/978-90-481-8569-6.

Pauly, K., Goossens, R. and De Clerck O., 2010. Mapping coral-algal dynamics in a seasonal upwelling area using spaceborne high resolution sensors. ESA living planet meeting. 8pp.

Pilgrim, D.A., Parry, D.M., Jones, M.B. and Kendall, M.A., 2000. ROV image scaling with laser spot patterns. Underwater Technology, 24: 93-103.

Provan, J., Booth, D., Todd, N.P., Beatty, G.E. and Maggs, C.A., 2008. Tracking biological invasions in space and time: elucidating the invasive history of the green alga *Codium fragile* using old DNA. Diversity and Distributions, 14(2): 343-354.

Rhoads, D.C., Ward, R., Aller, J.Y., and Aller, R.C., 2001. The importance of technology in benthic research monitoring: looking back to see ahead. In: Aller, J.Y., Woodin, S.A., Aller, R.C. (Eds.), Organism–Sediment Interactions. Belle W. Baruch Library in Marine Science, vol. 21. University of South Carolina Press, Columbia, SC, pp. 1-18.

Robbins, B. and Bell, S., 1994. Seagrass landscapes: a terrestrial approach to the marine subtidal environment.Trends in Ecology and Evolution, 9: 301-304

Schaffelke, B., Smith, J.E. and Hewitt C. L., 2006. Introduced macroalgae. A growing concern. Journal of Applied Phycology, 18: 529–541. doi: 10.1007/s10811-006-9074-2

Shimoni, M., Sirjacobs, D., Borges, A., Frankignoulle, M., Acheroy, M., Djenidi, S., Chou, L. and Vyvermans, W., 2003. Airborne hyperspectral potential for coastal biogeochemistry of the Scheldt estuary and plume. Paper presented at Proceedings of the The 2nd EARSeL Conference on Hyperspectroscopy, Hirchen (Munich), Germany, May 2003.

Sinclair, D., Williams, B. and Risk, M., 2006. A biological origin for climate signals in coral trace element "vital effects" are ubiquitous in scleractinian coral skeletons. Geophysical Research Letters, 33: L17707 10.1029/2006GL02718£3.

Singh, H., Can, A., Eustice, R., Lerner, S., McPhee, N., Pizarro, O. and Roman, C., 2004. Seabed AUV Offers New Platform for High-Resolution Imaging. EOS, Transactions, American Geophysical Union, 85 (31): 289, 294-295. doi:10.1029/2004EO310002

Sirjacobs, D., 2002. Matlab designed Image analysis software for detection of *Codium elisabethae* and calculation of population statistics. Erasmus report contribution to MAROV Project (Mapping of Marine Habitats of the Azores using Robotic Ocean Vehicles) at Department of Oceanography and Fisheries, University of the Azores, Faial Island. Université de Liège. 31pp + Annexes. http://www.horta.uac.pt/projectos/marov/reports/Final/Damien_2002.pdf

Sirjacobs, D., Shimoni, M., Borges, A., Chou, L., Frankignoulle, M., Vyvermans, W., Djenidi, S. and Acheroy, M., 2003. Biogeochemistry of the Scheldt estuary and plume. Paper presented at CASI-SWIR Workshop, Bruges, Belgium, September 2003.
http://hdl.handle.net/2268/28266

Solan, M., Germano, J.D., Rhoads, D.C., Smith, C., Michaud, E., Parry, D., Wenzhöfer, F., Kennedy, B., Henriques, C., Battle, E., Carey, D., Iocco, L., Valente, R., Watson, J. and Rosenberg, R., 2003. Towards a greater understanding of pattern, scale and process in marine benthic systems: a picture is worth a thousand worms. Journal of Experimental Marine Biology and Ecology, 285: 313–338. doi:10.1016/S0022-0981(02)00535-X

Tempera, F., and Santos, R.S., 2005. Projects and tasks related to Habitat Mapping. Recent and ongoing work in the Azores (Portugal). Presented at the 2005 meeting of the "ICES Working Group on Marine Habitat Mapping", Bremerhaven (Germany).

Tempera, F., 2009. Benthic habitats of the extended Faial Island shelf and their relationship to geologic, oceanographic and infralittoral biologic features. PhD thesis, 23-06-09, University of St. Andrews, UK, 341 pp.
http://hdl.handle.net/10023/726

Theriault, C., Scheibling, R., Hatcher, B. and Jones, W., 2006. Mapping the distribution of an invasive marine alga (*Codium fragile* spp. *tomentosoides*) in optically shallow coastal waters using the compact airborne spectrographic imager (CASI). Canadian Journal of Remote Sensing, 32 (5): 315-329.

UNESCO, 1998. CARICOMP - Caribbean coral reef, seagrass and mangrove sites. Coastal region and small island papers 3, UNESCO, Paris, xiv+347 pp.
http://www.unesco.org/csi/pub/papers/papers3.htm

Vaissière, R., Fredj, G. And Falconetti, C., 1972. Contributions à l'étude bionomique de la Méditerranée occidentale – Côte du Var et des Alpes Maritimes, Côte Occidentale de Corse. Unpublished, as illustrated in Bay (1978).

Verbruggen, H., Tyberghein, L., Pauly, K., Vlaeminck, C., Van Nieuwenhuyze, K., Kooistra, W., Leliaert, F. and De Clerck, O., 2009a. Macroecology meets macroevolution: evolutionary niche dynamics in the seaweed *Halimeda*. Global Ecology and Biogeography, 18: 393-405.

Verbruggen, H., Pauly, K., Tyberghein, L., Mineur, F. and Maggs, C., 2009b. Niche models of invasive seaweed species: predicting spread and areas at risk of suffering blooms, in: Mees, J.; Seys, J. (Ed.) (2009). VLIZ Young Scientists' Day, Brugge, Belgium 6 March 2009: book of abstracts. VLIZ Special Publication, 41: 18 pp.

Zajac, R.N., 1999. Understanding the sea floor landscape in relation to impact assessment and environmental management in coastal marine sediments. In: Gray, J.S. (Ed.), Biogeochemical Cycling and Sediment Ecology, vol. 59. Kluwer Academic Publishing, Dordrecht, The Netherlands: 211-227.

5 - Benthic environmental conditions

Table of contents

5.1 Introduction and objectives

Both physical and chemical properties of benthic habitats are known to shape the distribution, structure and dynamics of benthic communities and populations in a very complex way (Pauly and De Clerck, 2010). Two reference sites were selected within the Monte da Guia SAC (Faial, Azores) for long term ecological monitoring of the macroalga *Codium elisabethae.* Although depth and substrate types were relatively similar between these two sites, they show important differences regarding their biological occupation, as suggested by the density, size structure and evolution of *Codium elisabethae* (see Chapter 7). This suggests the need to develop better understanding of the local physico-chemical factors structuring the benthic life in these two reference sites. In particular, the long term ecological understanding of the dynamic equilibriums of this important macaronesian macroalga requires a most complete and synthetic view on both general and site specific particularities of the seasonal benthic abiotic pressures it has to cope with.

The objectives of this study are 1) to describe the general seasonal cycle of benthic environmental constraints encountered in 20 meter deep reference reef habitats within the Monte da Guia SAC, 2) to check for potential differences of these abiotic constraints between the reference study sites of the Caldeirinhas and of Ponta Furada, and 3) to propose a first interpretation of the specificities of local hydrodynamic regimes justifying the differences of environmental parameters observed between both sites.

For these targets, numerous sensors were deployed in both sites on specifically designed docking stations for a one year monitoring program (summer 2004 - summer 2005). The parameters measured (temperature, currents, turbidity, photosynthetic active radiation, salinity, nutrients) were selected by considering 1) notions which are potentially known to affect macroalgae population distribution and dynamics (Leliaert and Coppejans, 2004) and 2) practical constraints on sensor availability and deployment.

Specifically, this chapter aims mainly at producing a synthetic description of the benthic environmental "weather" found at both reference sites, in support to the qualitative interpretation of *Codium elisabethae* population dynamics and site specific differences in autecology (chapter 8). In addition, this chapter aims at preparing an environmental database foreseeing future multivariate analysis based on the integration of complementary data (from remote sensing, other benthic sensors, CTDs, recent physical models, see chapter 2), and on the extraction of various multi-temporal averages consistent either with the proposed ecological seasons based on growth rate (see chapter 7, section growth rate) either corresponding strictly with the given seabed mosaicking time series.

5.2 Material and methods

5.2.1 Sensor deployment and data gaps

During the summer 2004, 3 temperatures sensors « Minilog » were simultaneously installed in each Caldeirinhas and Ponta Furada sites for a 28-days record period. The 6 sensors were fixed to the rocky substrate along the long dimension of each imagery site). These sensors were logging temperature at 5 minutes intervals, from 16/07/04 to 11/08/04. With their high temporal and spatial resolution configuration, these six temperature sensors provided a first proxy indicator of the interaction between hydrodynamics patterns (thermocline, currents) and the seabed habitats in both sites.

Two identical docking stations were conceived specifically to receive any of the available sensor's shapes, to resist important wave impacts expected in winter, and to be displaced to other study sites for further investigations whenever appropriate. The preparation and installation of these docking stations with complete sensor packages took place in summer 2004.

The following sensors were installed at the study sites of Caldeirinhas and Ponta Furada (Fig. 1): one Nortek Aquadopp three-dimensional open water acoustic current meter equipped with integrated temperature and pressure sensors and one additional external OBS-3 turbidity sensor (data of all sensors are stored within the internal memory of the Aquadopp); one Aanderaa RCM9 two-dimensional acoustic current-meter equipped with integrated temperature, turbidity, pressure and conductivity sensors (data of all Aanderaa sensors are stored within the internal removable Data Storage Unit DSU 2990); two LICOR-192 Photosynthetic Active Radiation (PAR) sensors with specifically designed tanks sheltering autonomous batteries and data logging units.

Figure 1 – Sensors installed on docking stations in Caldeirinhas (left: RCM9 current-meter and PAR sensor) and in Ponta Furada (right: Aquadopp 3D, PAR and OBS turbidity sensor).

These sets of sensors were installed at proximity (about 20 meters) of the populations monitored by visible imagery. Their deployment lasted from august 2004 until end august 2005 in Ponta Furada and to end September 2005 in Caldeirinhas (details in Annex 10.1). The monitoring was planned as 4 cycles of 3 months, in order to respect the limitations of internal memories and battery life time, as well as minimal technical revision in laboratory. More frequent *in situ* cleaning operations was conducted whenever possible, and during other imagery or sampling dives. General sampling interval time was set to 20 minutes, allowing to resolve tidal cycle. Thus the 20 minutes averaged parameters recorded for both sites are current, pressure, temperature, turbidity and PAR, with an extra record of conductivity for the RCM9 current-meter (in the Caldeirinhas for the 3 first cycles), and an extra record of wave amplitude and directional spectrum every 3 hours (based on 3 minutes of 1 Hz data) for the Aquadopp 3D current-meter (in Ponta Furada for the 3 first cycles). During the last deployment cycle, the sensors were switched between sites to check for general artifacts due to sensor type.

Due to numerous technical difficulties in underwater environment, the data produced by the sensors are not always continuous time series. Problems in data acquisition resulted from various occasional battery lifetime shortage, problems in some of the new configuration software tested, sensitivity of electrical connections within specifically designed tanks, excessive biofouling, and even damage of professional cable caused by numerous bites from the curious fish *Balistes carolinensis* (Groy trigger fish, Peixe Porco). As consequence, the monthly averages and raw data seasonal cycles presented here are not continuous. The multivariate reconstruction of filtered but continuous cycles by statistical methodologies based on complementary signals from other sources is foreseen for other research objectives, but might require some complex modelling including high resolution wave propagation model, but comparison of available raw data was preferred at this stage.

5.2.2 Nutrient concentration in the seawater above seabed

In order to detect variations of nutrient content (total nitrates NO_3^- and nitrites NO_2^-; phosphates PO_4^{3-} and ammonium NH_4^+) in the water surrounding the studied populations, a total of 99 free water samples were taken at proximity of the seabed in the two sites. 41 sea water samples were collected in the site of Ponta Furada (12 in winter and 29 in summer), and 58 samples in the site of Caldeirinhas (26 in winter and 32 in summer). For each of these, 3 water samples replicates were collected and analyzed to improve precision.

Water samples were collected at a height of 20 cm above seabed over the study sites with syringes. In order to limit changes of nutrient content in the water samples before chemical analysis, syringes were equipped with a filter and a 20 cm pipe, pumping was made while progressing slowly in unperturbed bottom water layer, and syringes were quickly brought back to the surface and cooled in dark and cold conditions in ice pack box till the laboratory. They were then filtered again with individual new filters and frozen at -18°C for transportation till STARESO station (Corsica) where nutrient analysis was carried out with a SAN SKALAR automatic measurement chain. This system proceeds with automatic injection of colorimetric reactives into 3 channels holding the water sample (one for each nutrient), followed by a spectrophotometric measure.

5.3 Results and discussions

5.3.1 Temperature

Globally, the instantaneous values of temperature range from 16°C in March to 23 °C in September (Fig. 2). The monthly mean temperature values tend to be lower in the Caldeirinhas than in Ponta Furada site throughout the year, with a difference ranging from 0.1°C during the period December-March up to 1°C in August and October (Fig. 2).

As the site depths (+/-20 meters) correspond to the position of the seasonal thermocline (see chapter 2), a thermic oscillation in phase with the tidal signal can be detected during the stratification period (Fig. 2), which appears to affect these benthic sites from mid-May to early November (see monthly variances in table 1 and range of monthly mean values in figure 3). The thermic cycle is affected by the tidal amplitude modulation as temperature variations are higher during the periods of important tides.

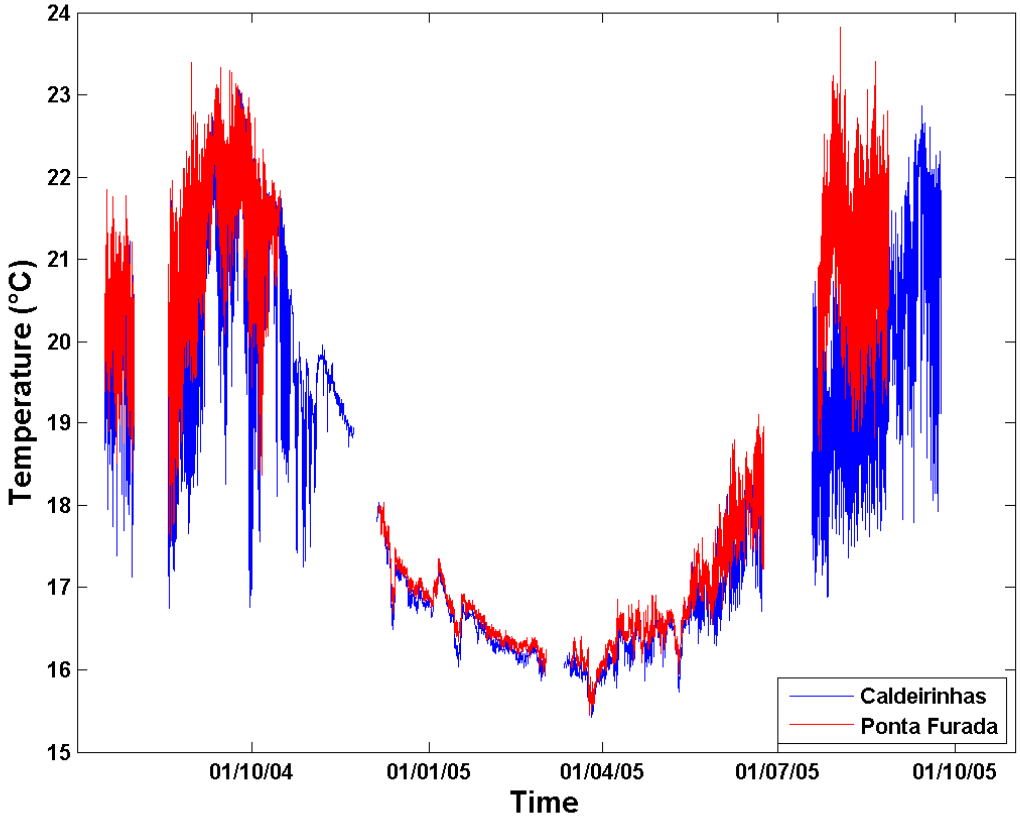


Figure 2 – Seasonal evolution of 20 minutes averaged water temperature at seabed in the study sites of Caldeirinhas (blue) and Ponta Furada (red).

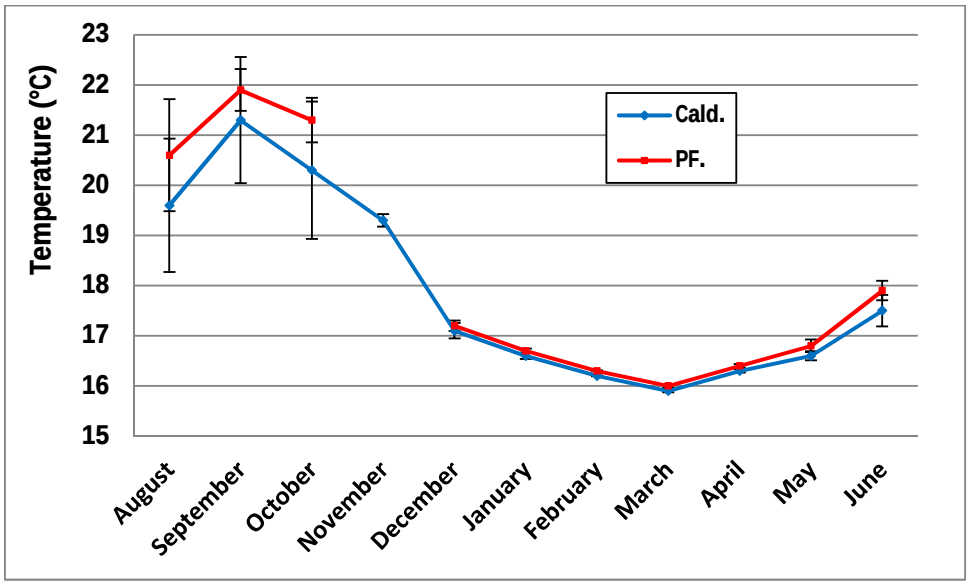


Figure 3 – Seasonal evolution of monthly mean water temperature at seabed in the study sites of Caldeirinhas (dotted line) and Ponta Furada (continuous), with monthly standard deviation.

Table 1 - Comparison of temperature monthly means and variances in both sites.

Month	Mean T°C		Max.T°C		Min T°C		Variance	
	Cald	PF	Cald	PF	Cald	PF	Cald	PF
August	19.6	20.6	22.1	23.4	16.7	17.6	1.329	1.117
September	21.3	21.9	23.1	23.3	16.7	19.5	1.257	0.417
October	20.3	21.3	22.2	22.7	16.9	18.3	1.369	0.446
November	19.3		19.9		17.9		0.124	
December	17.1	17.2	18	18	16.5	16.7	0.155	0.105
January	16.6	16.7	17.2	17.3	16	16.3	0.063	0.042
February	16.2	16.3	16.4	16.5	15.8	16	0.008	0.006
March	15.9	16	16.2	16.4	14.4	15.4	0.027	0.039
April	16.3	16.4	16.7	16.9	15.8	16	0.035	0.034
May	16.6	16.8	17.9	18	15.7	15.9	0.089	0.127
June	17.5	17.9	18.6	19.1	16.5	16.7	0.313	0.194

During stratified periods, the temperatures measured in the Caldeirinhas site show larger amplitudes than those in Ponta Furada, while the minimal values are almost always lower than in Ponta Furada (Fig. 4). These differences can be explained as resulting from the topography of the Caldeirinhas site which, in addition of being located 2 meters deeper, is in close proximity with an underwater cliff falling down to more than 100 meter deep (Figs. 5 and 10 in chapter 2), allowing entry of deeper colder waters during spring tide in the lower basin of the external Caldera, whereas the site of Ponta Furada is located in a general regular topography characterized by a weak general slope (Figs. 5 and 11 in chapter 2).

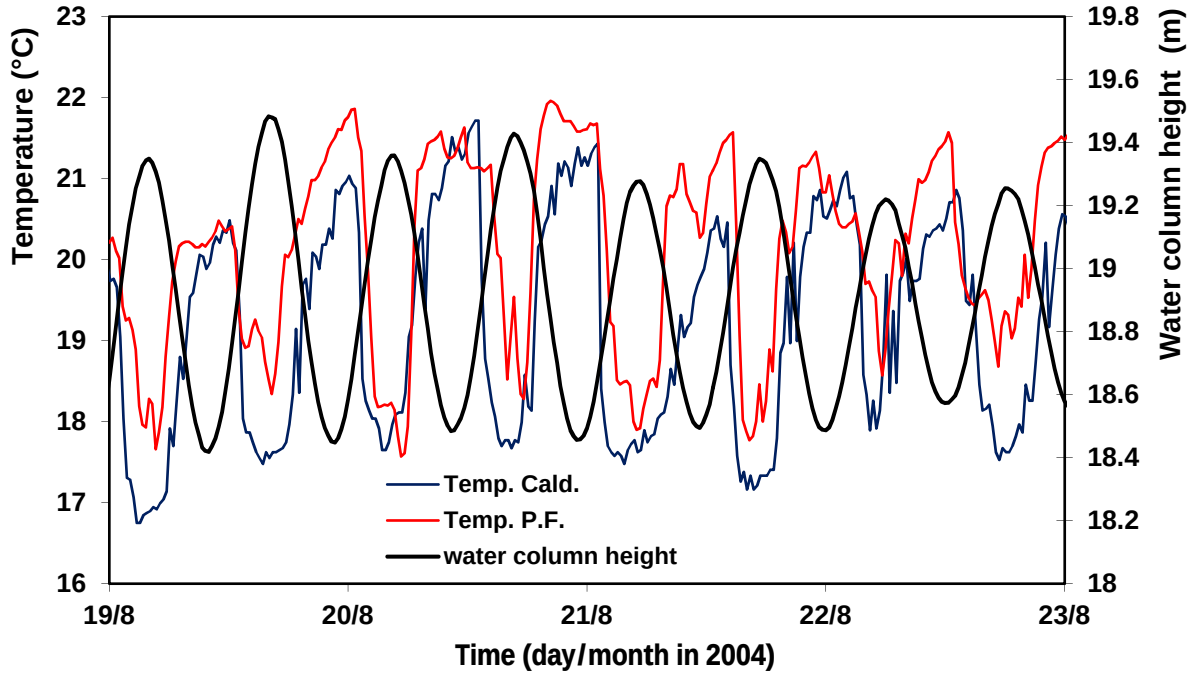


Figure 4 – Clear influence of the tidal cycle on the bottom water temperature in the Caldeirinhas and Ponta Furada sites, during the stratified summer period.

Occasional short-lasting but intense temperature reductions can be observed at the Caldeirinhas site, probably resulting of a combined effect of both thermocline oscillation and local upwelling circulation induced by particular configurations of wind, waves and tide.

Based on Minilog temperature data collected in summer 2004, the evolution of water temperature can be considered homogeneous in space at the scale of each site as no significant differences could be detected between the signals of sensors installed in each site. This means no sub-site scale temperature gradients could be detected within the sites due to any potential localized current effect. Consequently the mean of the three measures is considered as temperature of each site hereafter. The 5 minutes resolution look at summer temperature shows also the dominant effect of the tidal upward and downward movement of a summer stratified water column on the temperature signal experimented by the benthos biota in these sites (Fig. 5). During the summer period studied with Minilog sensors, maximum temperatures are generally less different between sites than minimum temperatures, which are frequently clearly lower in Caldeirinhas during high tide phase.

The water presents at bottom can be described as generally colder in the site of Caldeirinhas (mean difference of -0.72 °C), with instantaneous differences ranging from about –4°C to +1°C for the Caldeirinhas site when compared to the site of Ponta Furada (Fig. 6).

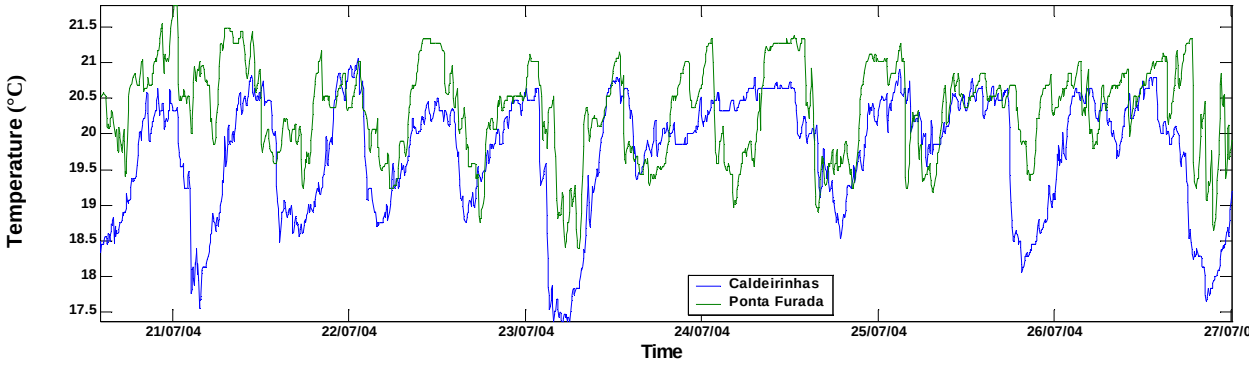


Figure 5 – Influence of the tidal cycle on the bottom water temperature in the Caldeirinhas and Ponta Furada sites measured at 5 minutes interval (Minilogs).

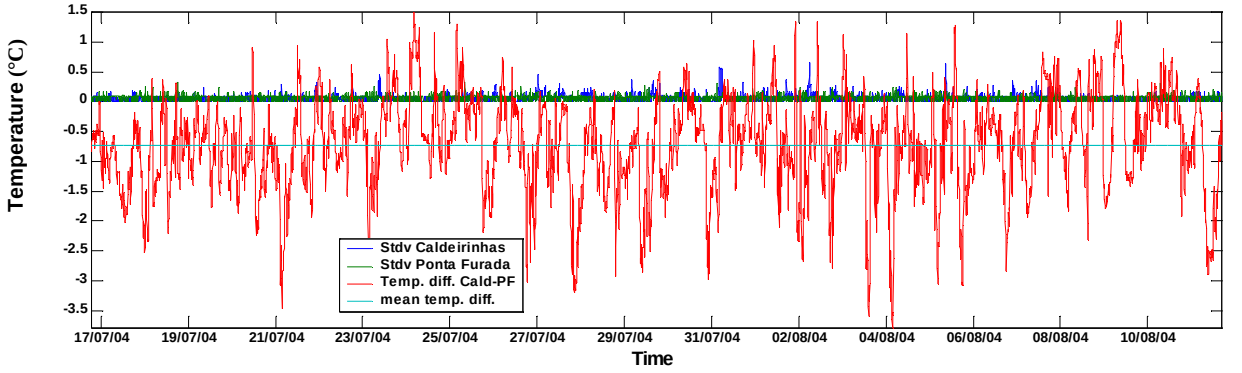


Figure 6 - Difference of bottom temperature between Caldeirinhas and Ponta Furada benthic sites measured at 5 minutes interval with Minilogs (red), and mean difference (light blue).

The phase of decreasing temperature is generally very sharp in the Caldeirinhas, with up to 3°C cooling in sometimes just 20 minutes (Fig. 5). This fast and intense temperature dropping measured by the Minilog sensors also suggests that the Caldeirinhas site is frequently completely occupied by a massive input of "lower or below-thermocline" waters during the stratified periods, and anyway generally more influenced by these waters than the Ponta Furada site, probably as much a consequence of the local interaction between tides and topography than of its 2 meters lower depth. As a matter of fact, in the Caldeirinhas site, the rising tide dynamics can bring deeper cooler waters to fill in the two calderas. This deeper cooler water is available just in front of the mouth of the outer caldera. Both the interaction of tidal dynamics and of northern winds with the specific local topography are suggested to induce a local upwelling mechanism at the mouth of the calderas. Colder water arrival in the calderas can also be justified by the presence of the larger upwelling mechanism described with CTD data at the proximity of this site, as a result of tidal movements over the Faial Pico channel (Bashmachnikov and Martins, 2006; Bashmachnikov, personal communication; Tempera, 2009). Comparatively, in Ponta Furada, the rising tide can only bring up to the site the water of a more uniformly mixed water column, as this site is less influenced by the south Faial Pico channel upwelling and as the general surrounding topography is a progressive slope till further off shore.

5.3.2 Currents

5.3.2.1 Typical current patterns and related forcing

The current patterns observed in the site of Ponta Furada reflect a combination of the predominant neap and spring tidal alternative components oriented in the east-west direction, superimposed on the general along shore currents leaning eastwards (Fig. 7). During raising spring tide, the current is heading east and reach its maximum speed during the middle of the raising phase of the tide and minimal value at high tide. Inversely the current are oriented westwards during descending phase and reach their maximum speed at the middle of this lowering tide followed by minimal speed at neap tide. The observed intensities are affected by the modulation of the tidal amplitude.

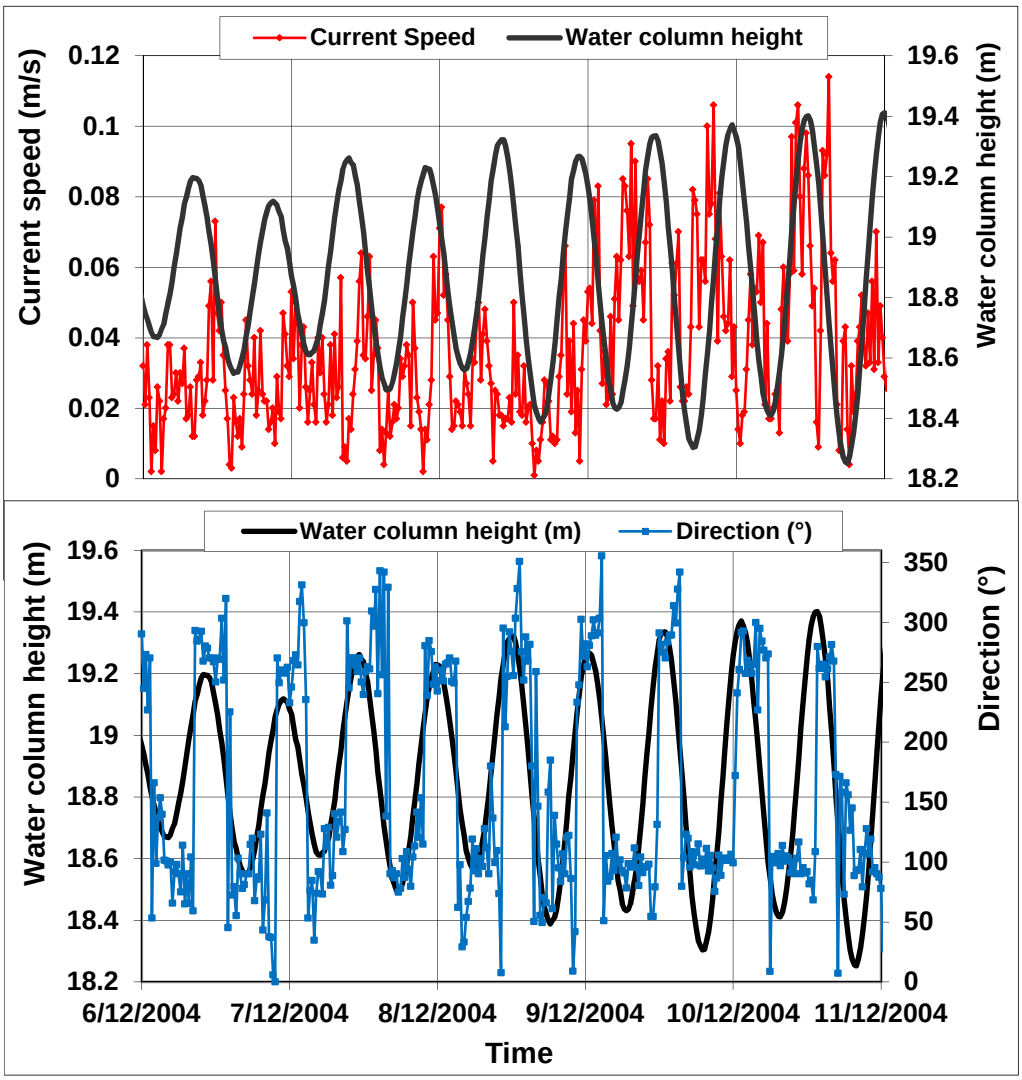


Figure 7 – Typical neap and tide currents in Ponta Furada site during calm conditions, showing clear tidal phase of current direction (blue) with water level cycle (black).

In the Caldeirinhas site, both the general along shore southern Azorean current and the tidal currents are affecting local current patterns in a much more complex way as a consequence of the relative isolation of the small bays constituted by the Caldeirinhas, and of the close proximity of the Faial-Pico channel entry. The dominant current dynamics observed there is rather clearly reflecting the forcing by intense waves heading in the Northern sector or by local complex hydrodynamic interactions provoked by the prominent and abrupt topography of the Caldeirinhas located on the way of the intensified coastal currents entering the Pico-Faial channel. These later interactions are not addressed in the context of this work but would deserve an integrative modelling study at the scale of the central group islands (Faial; Pico; São-Georges) and implying all available physical data and recent high resolution bathymetry for the benefit of close to shore benthic and pelagic habitat ecological studies.

When specific forcing conditions are united (strong wind and/or waves), the usual tidal temperature fluctuation can disappear in the Caldeirinhas and is replaced by a rather constant warmer temperature, as already seen in the minilog temperature data on the 23-24 July 2004 (Fig. 5) and confirmed for instance on the 24-26 September 2004 in regard to measured current and waves (Fig. 8).

This suggests the installation of a particular circulation pattern within the calderas, with warmer surface waters being forced to enter the calderas in the surface, inducing a bottom return current bringing mixed waters from the shallow inner caldera towards the mouth of the outer caldera via the 3 canyons linking the two basins. This situation can prevent for a while the deeper cold water from reaching the site of Caldeirinhas as it would do during usual raising tidal phase.

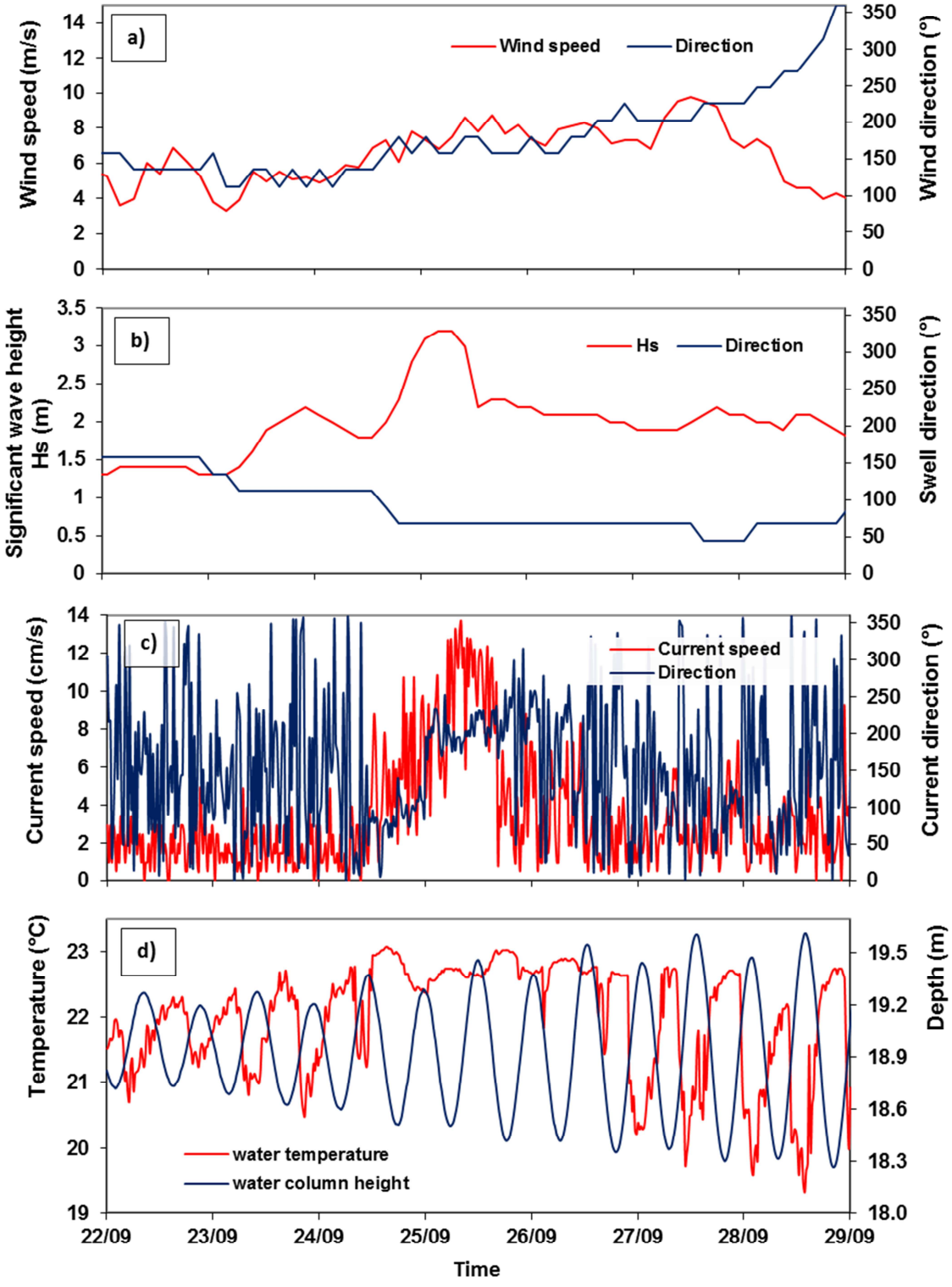


Figure 8 – Installation of a bottom return current in the Caldeirinhas in relation with an intense NNE swell: a) wind speed (red) and direction (blue) ; b) wave height (red) and direction (blue); c) current speed (red) and direction (blue); d) temperature tidal cycle (red) interrupted during 4 tidal cycles of water column height (blue).

5.3.2.2 Monthly mean and maximum integrated current

The monthly mean of the 20 minutes averaged current speed observed in Ponta Furada was generally higher than in the Caldeirinhas, except for the month of March 2005 (Fig. 9). In the Caldeirinhas, the monthly mean speed are higher in winter than in summer, whereas the highest values of available current data are observed in spring and summer in Ponta Furada (although some data are missing to affirm it). During winter period (except March), the monthly mean speeds that can be compared show a less pronounced difference between both sites than during the summer period. The much higher mean speed observed in the Caldeirinhas in March compared to other months is related to a very intense hydrodynamic event which forcing and principle was explained in section (25/03/05, swell of more than 5 m significant height heading NNE in dominant direction, provoking maximum instantaneous speeds of 2 m/s in Ponta Furada and intense 60 cm/sec return currents in Caldeirinhas).

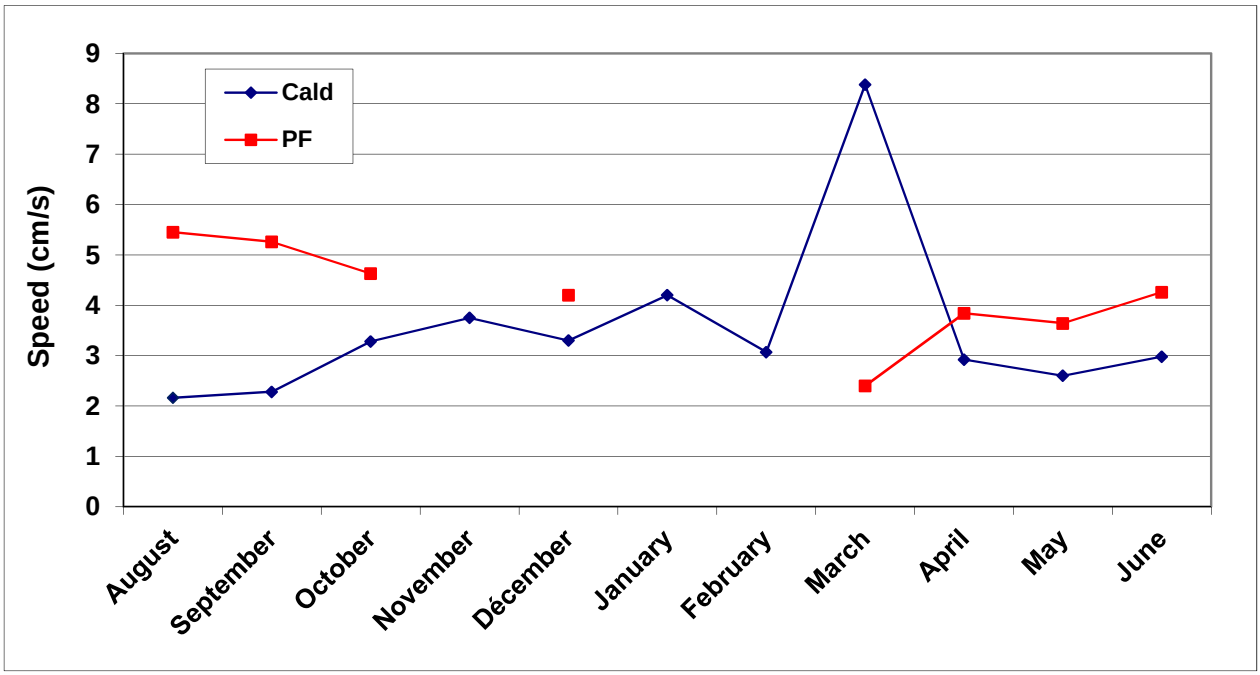


Figure 9 – Monthly mean current speed at bottom in both sites (August 2004 – June 2005)

The monthly maximum of the 20 minutes averaged current speed was higher in Caldeirinhas than in the Ponta Furada during months of October, March and April while the opposite was observed for the months of August and September (Fig. 10). In May and June, these values are similar.

The occasional much higher maximum speeds detected in Caldeirinhas are mostly resulting from short-lasting extreme events rather than reflecting frequent average situations. For instance, during the month of October, for which the monthly maximum speed in Caldeirinhas was double than in Ponta Furada (Fig. 10), the 20 minutes averaged speed raised higher than 10 cm/s only on 35 occasions in the Caldeirinhas but on 66 occasions in Ponta Furada.

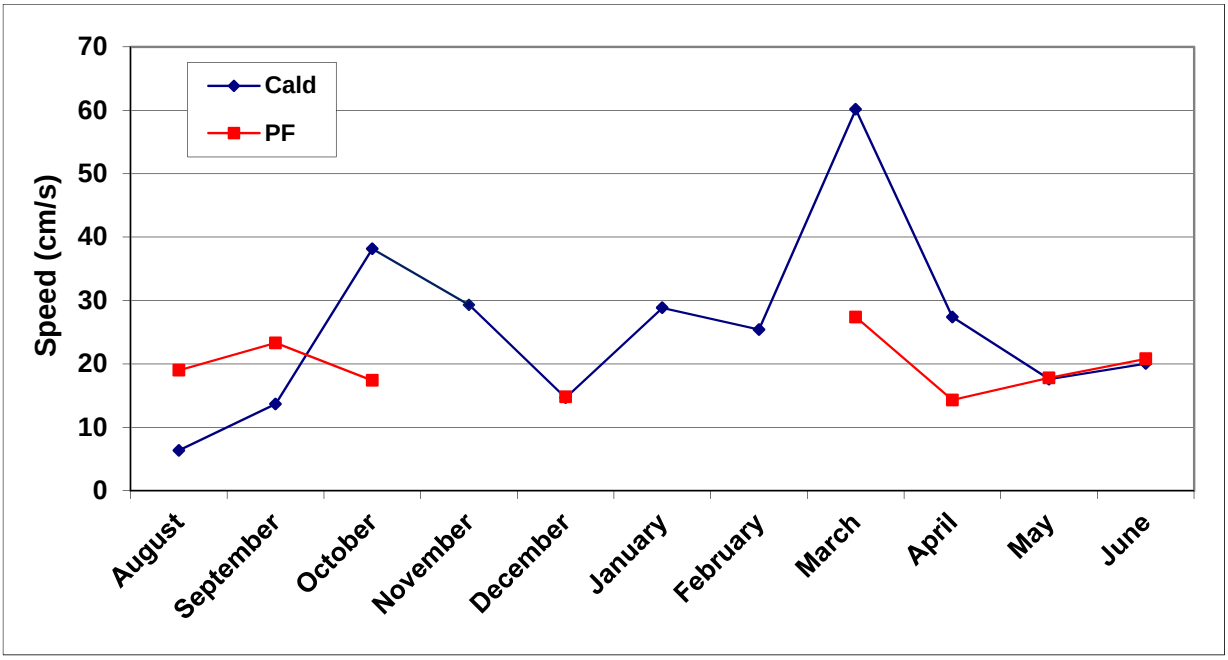


Figure 10 – Monthly maximum of 20 minutes averaged current speed at bottom in both sites (August 2004 – June 2005)

5.3.2.3 Hodographs and direction histograms

Typical differences of mean current and general advection affecting the study sites are illustrated for 2 characteristic periods (August and December) by hodographs (integrative current plots; Figs. 11a-b and 12a-b) and direction histograms (Figs. 11c-d and 12c-d). For the other months, we refer to work supervised in Youssef (2005).

In December, the current direction histogram (Fig. 11c-d) clearly shows the difference of current regimes between the sites, with most frequent currents heading south in the Caldeirinhas in agreement with the local induced circulation explained above (section 5.3.2.1), while dominant directions are clearly oriented east and west in Ponta Furada, hence reflecting the dominant along shore tidal current components. The maximum 20 minutes averaged speeds are of similar order for both sites (~= 15 cm/s), and the resulting directions (Fig. 11a-b) are clearly representing the bottom return current leaving the Caldeirinhas southwards toward the calderas mouth and the signature of the general eastern heading Azorean current in Ponta Furada site.

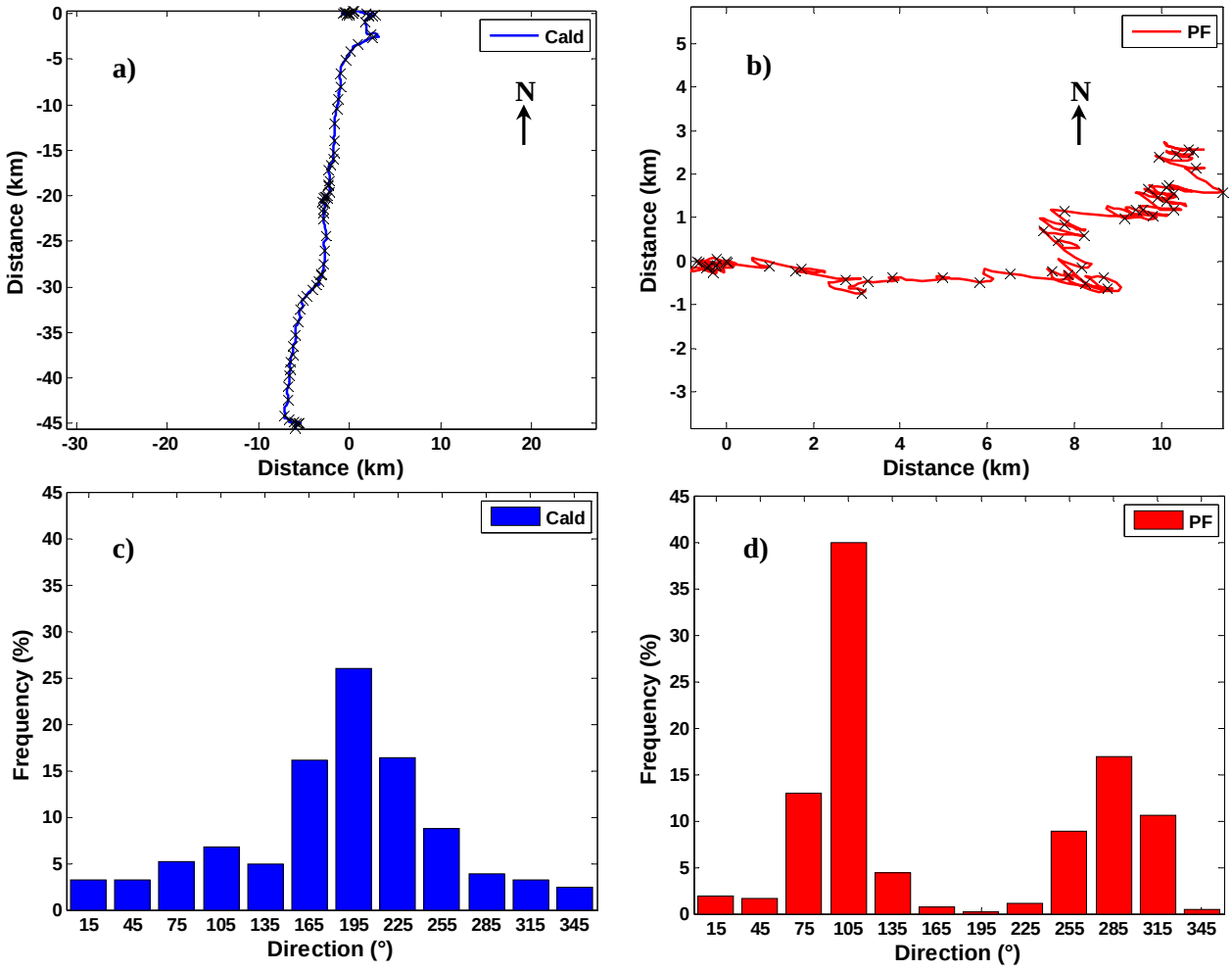


Figure 11 – Hodographs (a and b; with cross mark every 3 hours) and current direction histograms (c and d) for the month of December 2004 in Caldeirinhas (blue, left) and Ponta Furada (red, right).

In August, the basic winter dominating patterns are modified in both sites and less clear differences in dominant directions appear on the current direction histograms (Fig. 12c-d).

In Ponta Furada, the maximum 20 minutes averaged speed (19 cm/s) August is more important than in December (15 cm/s), whereas for the Caldeirinhas, this summer value is clearly lower (6 cm/s) than the winter one (15 cm/s). This suggests that a modification of the contribution of the general coastal current increases the values observed in summer in Ponta Furada, whereas the wave induced local circulation described for the Caldeirinhas is much less intense in summer, which is logical considering lower frequency and intensity of important wave events.

The resulting global water displacements (Fig. 12a-b) are both oriented south-east in summer. This change in direction in Ponta Furada suggest that a general bottom return current is active along this coast during this summer month, adding a southern component to the general eastern current. This southern component can clearly be seen on the Ponta Furada hodogram (Fig. 12b) during some of the tidal current reversal periods.

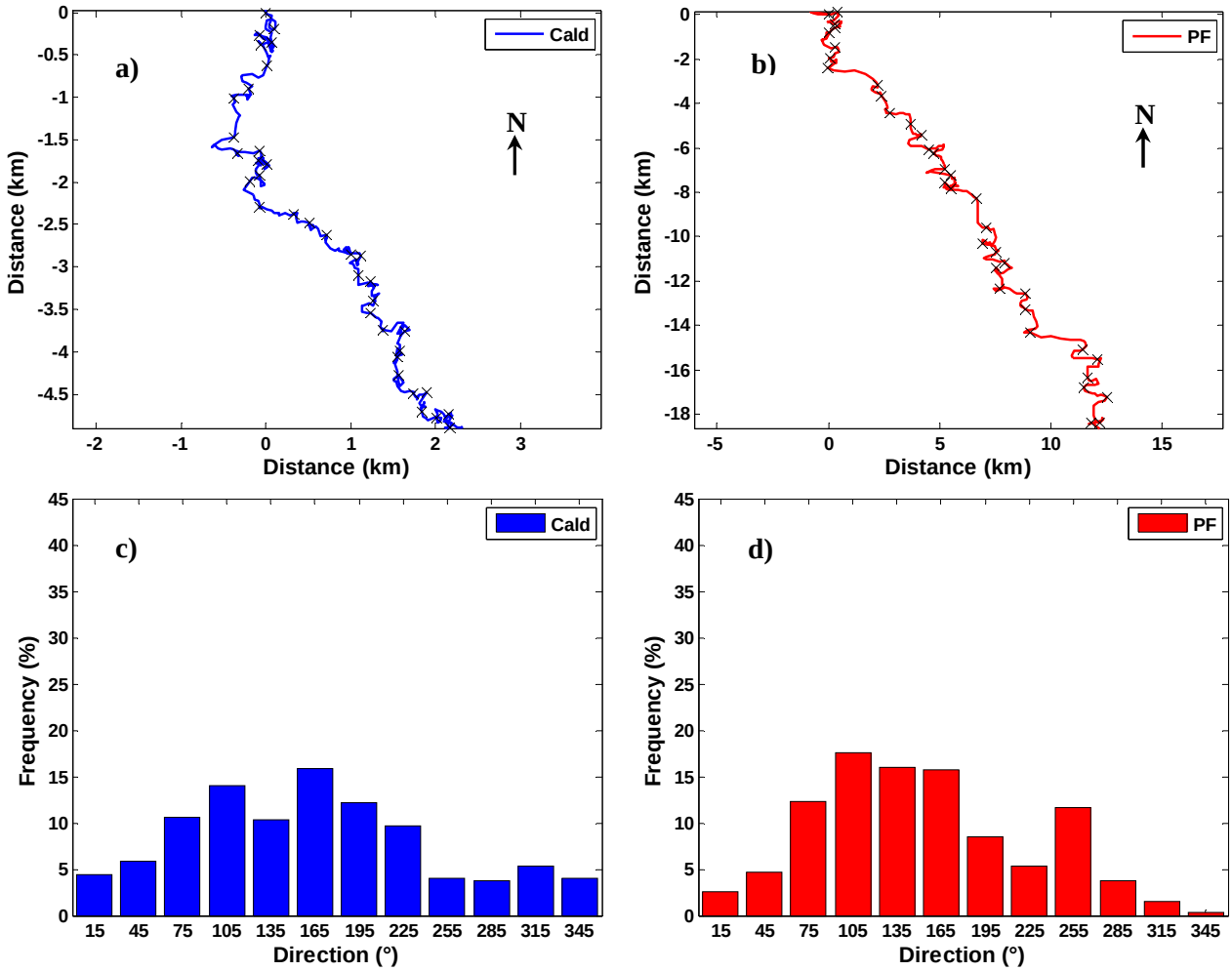


Figure 12 – Hodographs (a and b; with cross mark every 3 hours) and current direction histograms (c and d) for the month of August 2005 in Caldeirinhas (blue, left) and Ponta Furada (red, right).

5.3.3 Waves and instantaneous water movements

Based on the high frequency acquisition of wave and currents delivered by the Aquadopp sensor in the site of Ponta Furada (3 minutes at 1Hz every 3 hours), the maximum instantaneous horizontal current speed recorded at this 20 m deep seabed can be clearly linked with the significant wave height (Hs) computed from the corresponding record (Fig. 13). Significant wave height explains 80 % of the variability of maximum instantaneous speed recorded at bottom, showing that even for the site of Ponta Furada which is more exposed to coastal and tidal currents, the most intense drag effects on seabed organisms is the wave impact. This will obviously be the case also for the Caldeirinhas as average advection currents are of lower intensities than in Ponta Furada, and wave fields can occasionally be amplified in the Caldeirinhas.

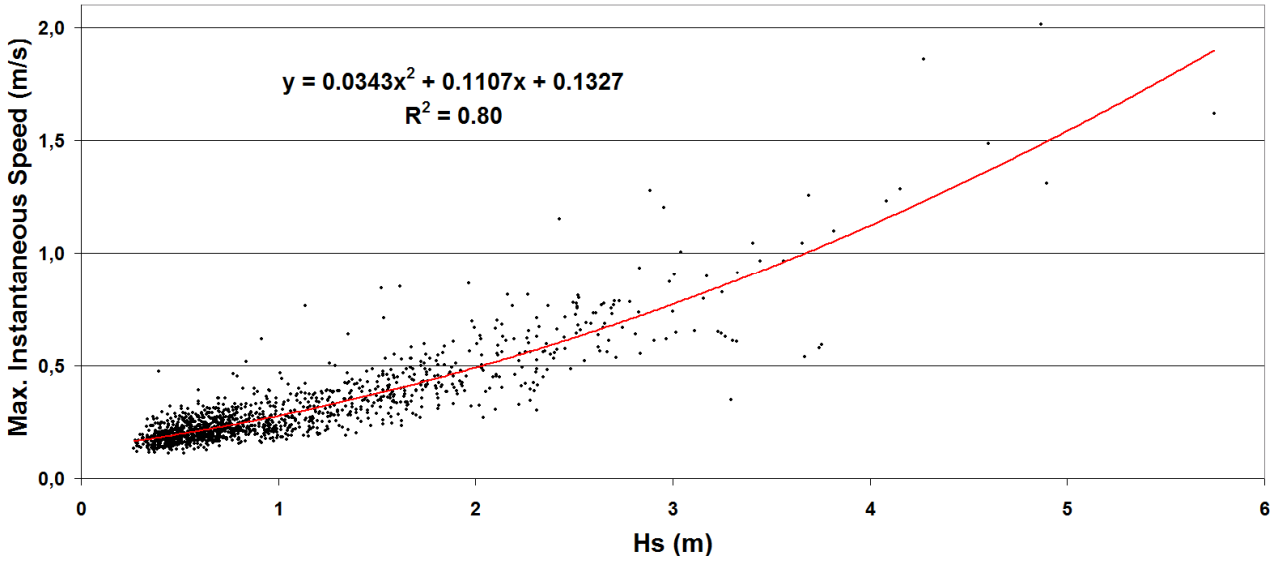


Figure 13 – Correlation between maximal instantaneous speed at bottom and significant wave height recorded in the site of Ponta Furada.

5.3.4 Turbidity

The monthly mean turbidity measured in Ponta Furada is much higher (range of 1 to 100 NTU) than in the Caldeirinhas (below 1 NTU) (Fig. 14). The evolution of turbidity followed on the occasion of intense storms (example in figure 15) shows that little intensification of the current (10 cm/s) can raise turbidity to high values in Ponta Furada (over 220 NTU), whereas more intense currents (60 m/s) in the Caldeirinhas can only raise turbidity to limited values (25-30 NTU). This difference of turbidity evolution is due to the presence of extensive sand fields at the proximity of the Ponta Furada reef site studied by opposition of the situation in the Caldeirinhas. Although this general difference of turbidity between the sites would tend to reduce more drastically the PAR reaching the seabed habitat of Ponta Furada, the higher shadowing effect of the surrounding topography and the slightly deeper situation of the Caldeirinhas do act in the opposite way and define predominantly the difference of PAR reaching these habitats.

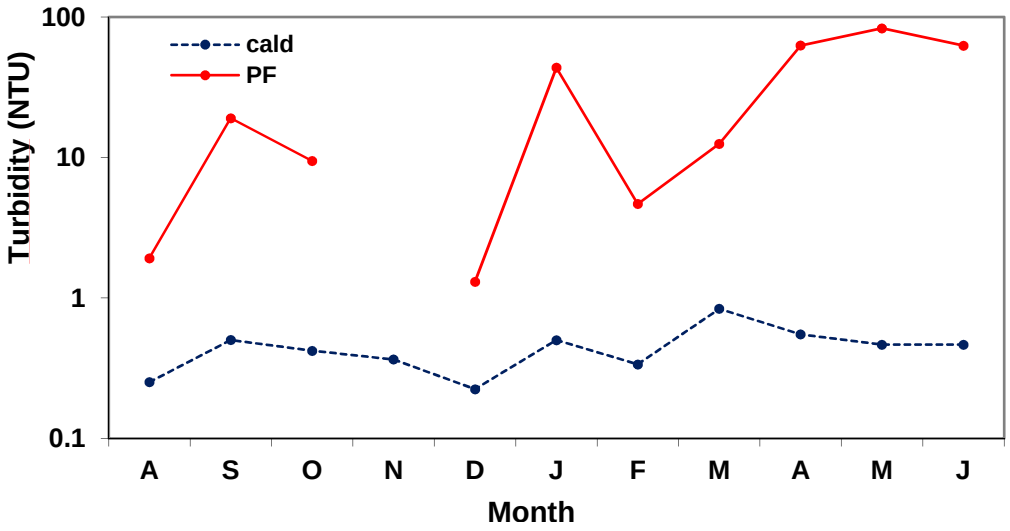


Figure 14 – Monthly mean turbidity observed in both sites from August 2004 to June 2005.

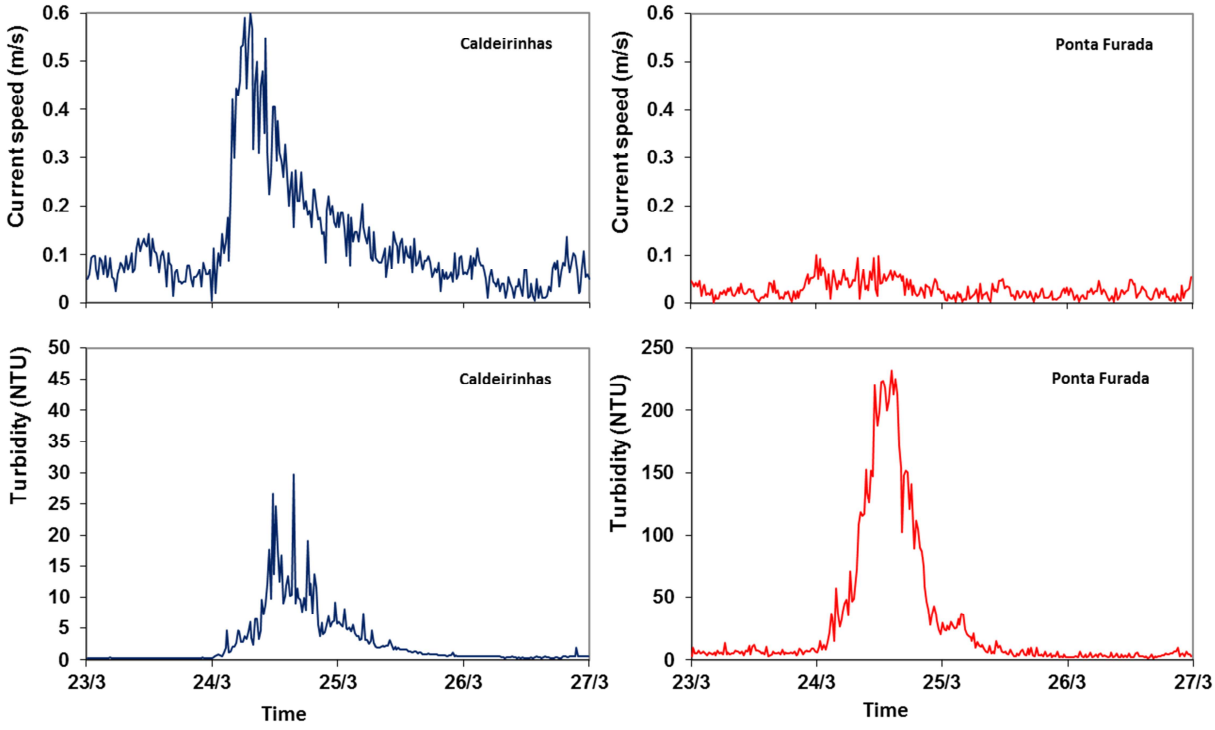


Figure 15 – Evolution of current speed (up) and turbidity (down) observed during a particular storm event (23-27/03/2005) in the Caldeirinhas (blue, left) and in Ponta Furada (red, right).

5.3.5 Photosynthetic Active Radiation (PAR)

The mean daily photon fluxes reaching these benthic habitats range globally from 1 to 7 mol/(m².d). These photon fluxes are higher in the Ponta Furada site than in the Caldeirinhas, both in winter and in summer (Fig. 16). The mean ratio of daily photon flux (mol/m².d) reaching Ponta Furada seabed habitat as compared to the Caldeirinhas habitat is of the order of 1.5. This difference is probably mainly due to the shadowing effect of the high crater slopes surrounding the Caldeirinhas seabed habitat on more than 270 degrees orientation, and to a lower extent to its 2 meters deeper situation.

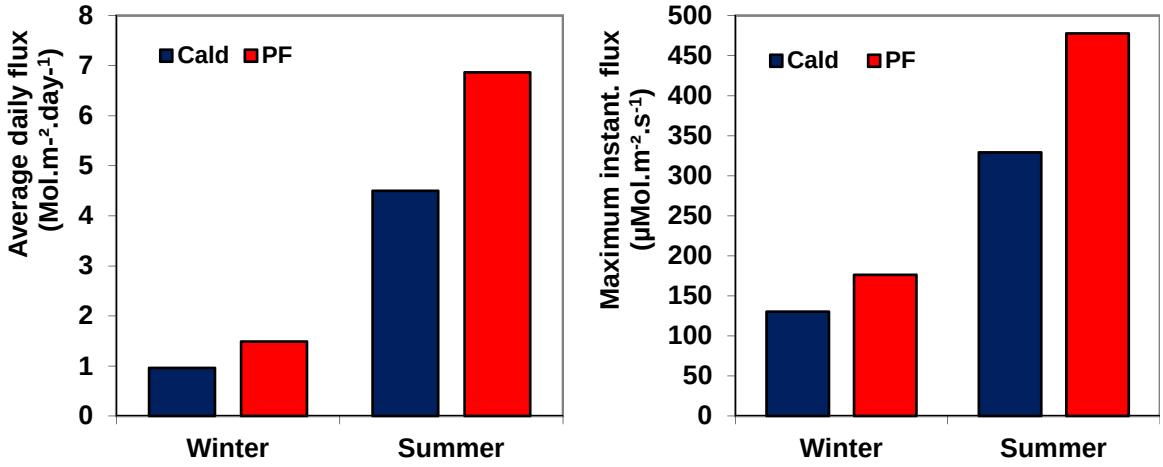


Figure 16 – Comparison of winter and summer daily mean photon fluxes (left) and maximal instantaneous photon fluxes (right) reaching seabed in the PAR waveband, for both sites.

Concerning the duration of non-null PAR light sensed at seabed (kind of local "photoperiod"), the measurements showed that the duration of this positive signal is shorter in the Caldeirinhas habitat as compared to the Ponta Furada site. This difference ranges from 20 minutes in summer up to 2 hours in winter, which is a logical modulation of the shadowing effect of local surrounding topography on direct solar radiation reaching sea surface above the sites, and subsequently on PAR reaching seabed. This shadowing effect can also be seen on figure 17 with lower instantaneous PAR fluxes and a faster light increase and reduction at dawn and nightfall in the site of Caldeirinhas.

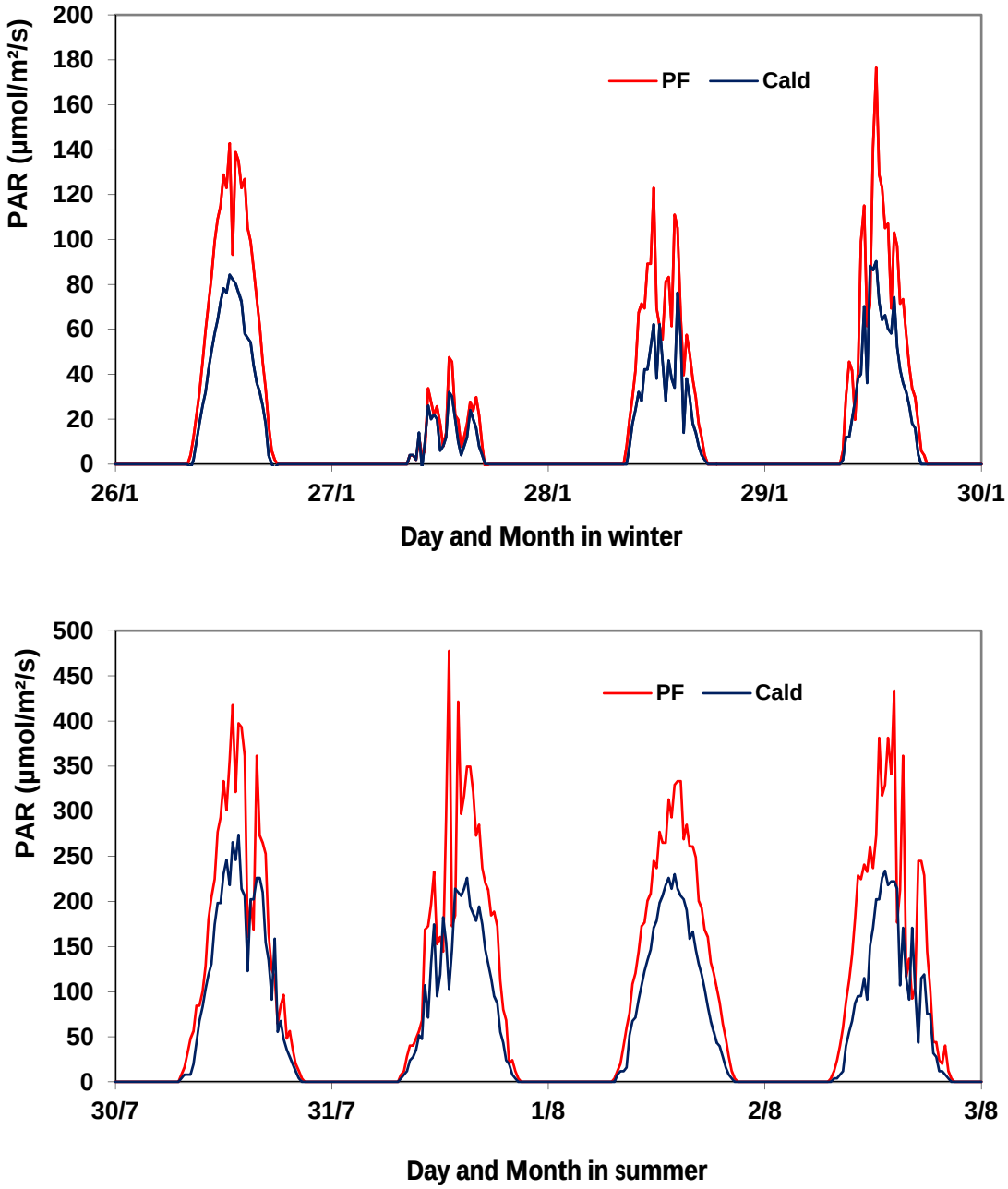


Figure 17 – Comparison of typical winter (up) and summer (down) instantaneous PAR photon fluxes reaching seabed, for both sites (year 2004).

5.3.6 Nutrients

Coastal waters in the south of Faial can be considered as an unperturbed oligotrophic environment, with global concentration ranges of 0.12 – 2.81 µM/l for nitrates, 0.13 – 2.23 µM/l for ammonium and 0.03 - 0.17 µM/l for phosphates. A high temporal variability of nutrient concentration is observed at proximity of the seabed, resulting from complex combined effects of recent specific hydrodynamic events (sediment re-suspension, upwelling), thermocline position according to the moment of the tidal cycle, origin of currents according to local small scale forcing, or intense raining events bringing organic and mineral soil compounds from slope erosions.

The frequency of the nutrient sampling carried out in this study could not resolve these dynamics and thus seasonal and site averages are probably somehow biased by under-sampling. Nevertheless, some significant information could be produced. Globally, while aggregating all 99 samples data for both sites, the summer concentrations appear significantly lower than the winter ones for all 3 nutrients considered.

The mean values of nitrate, ammonium and phosphate measured from these samples are illustrated in figures 18 and 19, revealing a clear seasonal trend of higher concentrations in winter than in summer for all nutrients and for both sites.

The higher winter values of nutrients can be explained by higher advection and diffusion of nutrients from deeper waters due to the absence of upper thermocline and to more intense mixing. The establishment of the summer thermocline isolates the surface water layer from this input, leading to a progressive reduction in nutrient concentration in summer due to uptake by primary producers.

Student t-tests reveal that for the site of Ponta Furada, winter nitrate and phosphate are significantly higher than in summer ($p=0.000024$ for nitrate; $p=0.000031$ for phosphate), while the seasonal difference is not significant for the ammonium. For the Calderinhas sites by opposition, only the ammonium is significantly higher in winter than in summer ($p=0.00117$), while nitrate and phosphate concentrations do maintain relatively high values in summer.

In winter, no significant differences in nutrient concentration could be detected between both sites, whereas in summer, the nitrate and phosphate concentrations are significantly higher in the Caldeirinhas sites than in Ponta Furada ($p=0.04814$ for nitrate; $p=0.00197$ for phosphate). This high nitrate and phosphate concentrations remaining in summer in the Caldeirinhas can be explained by several factors: 1) the local particular topography surrounding the site: the small semi enclosed bay facing an abrupt underwater cliff at the entry of the Faial-Pico channel can be occasionally subject to local deep water intrusion pushed by the local effects of waves, currents and winds in this particular topographical configuration. The entry of the main canyon at the edge of the outer Caldeirinhas (-35 m) is known by divers to be subject to very intense currents; 2) the proximity of the site to the southern entry of the Faial-Pico channel, an area identified as subject to local upwelling due to the interaction of tidal currents with the relatively shallow topography linking both islands (Bashmachnikov *et al.*, 2006 and Tempera, 2009).

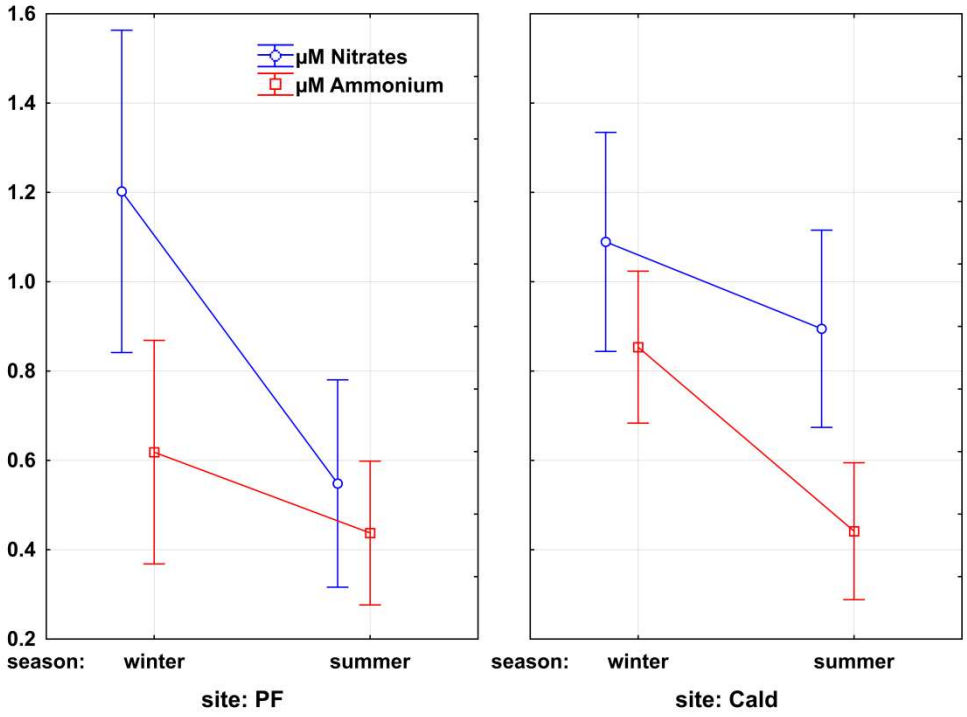


Figure 18 – Mean summer and winter nitrate and ammonium concentrations in the seawater above seabed, averaged for the sites of Ponta Furada (left) and Caldeirinhas (right); shown with 95% confidence intervals.

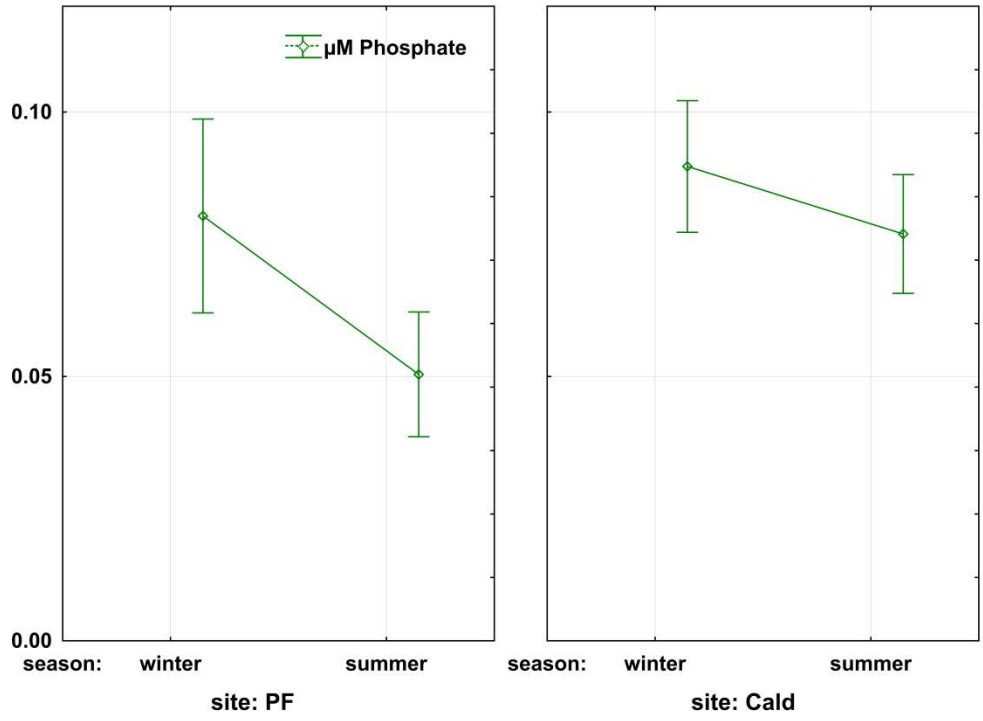


Figure 19 – Mean summer and winter Phosphate concentrations in the seawater above seabed, averaged for the sites of Ponta Furada (left) and Caldeirinhas (right); shown with 95% confidence intervals.

5.3.7 Salinity

The salinity observed in the Caldeirinhas ranged from 35 to 37, with an average value of 35.9. Intense salinity drop events are observed in the Caldeirinhas (Fig. 20), frequently in conjunction with important rain events (as obtained from windguru model), resulting from the intense run-off concentration effect along land slopes of the Caldeirinhas, or from the possible presence of groundwater spring fluxes at proximity of the sensor, which might be set in pressure by raising level in island lakes and ground water tables after intense rain events. Rain and cloud cover are resulting from large scale models and are relatively accurate on the rain occurrence, but not very precisely in time as they are not considering the island effect and even less local topography effects which might are an important parameter in the local rain distribution and occurrence.

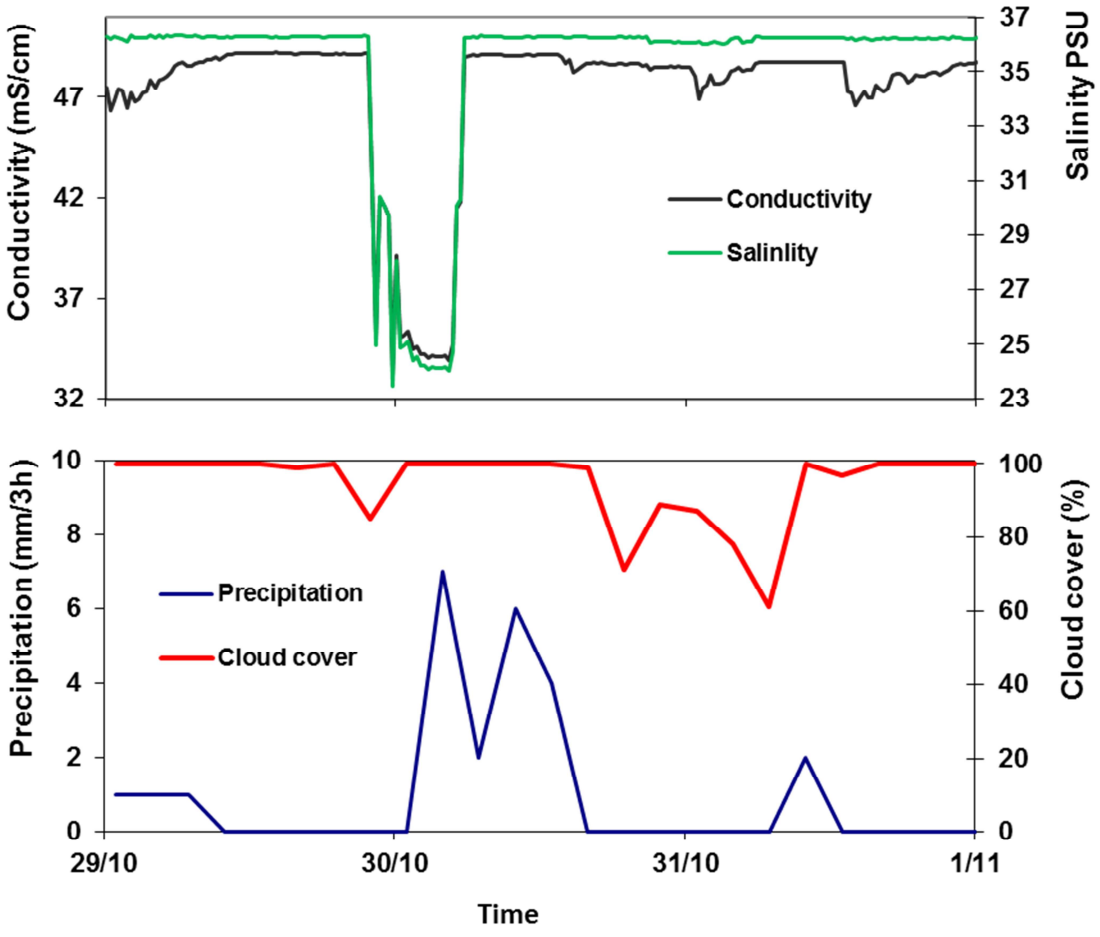


Figure 20 – Illustration of occasional conductivity and salinity intense reductions measured in the Caldeirinhas (up) in conjunction with intense rain events (down, data from regional model).

5.4 Conclusions

This research produced a one year synthetic seasonal description of benthic environmental forcing affecting two reference 20 m deep seabed habitats selected for long term macroalgal monitoring within the Monte da Guia SAC (Faial, Azores). The installation and regular maintenance of complete set of sensors during one year (summer 2004 - summer 2005) in both study sites allowed for the first time the compilation of an extensive multi-parameter database exploitable for any future studies on the coastal oceanography and benthic ecosystem processes affecting this Faialense coast. The environmental conditions encountered at the bottom can be summarized as follows:

- Current regime is mainly defined by typical East-West tidal currents superimposed on the general Azores Current in the Ponta Furada site. The site of the Caldeinrinhas is sheltered from this main coastal current and its complex local regime results from tidal inflow and outflow into the caldera's basins and from wave and wind induced return circulation displaying a dominant north-south component. Twenty minutes average current speed are generally lower in the sheltered Caldeinrinhas, except on the occasion of intense southern wind and wave forcing (up to 0.6 m/s). Maximum instantaneous speeds (2 m/s) affecting these 20m deep habitats do correspond to intense swell (Hs 5 m) impact.

- The instantaneous values of temperature range from 16°C in March till 23 °C in September. The monthly mean temperature values tend to be lower in the Caldeirinhas than at the Ponta Furada site throughout the year, with a difference ranging from 0.1°C during the period December-March up to 1°C in the period August-October. A thermal oscillation in phase with the tidal signal can be detected during the stratification period which affects these benthic sites from mid-May to early November. Various observations reveal that the Caldeirinhas site is frequently completely occupied by a massive input of “below thermocline” colder waters during the stratified periods, and is generally more influenced by deeper waters than the Ponta Furada site.

- The turbidity is much higher in Ponta Furada (range of 1 to 100 NTU) than in the Caldeirinhas (below 1 NTU) presumably due to the extensive sand fields present in the vicinity of the Ponta Furada reef site.

- The monthly mean daily photon fluxes reaching these benthic habitat ranges globally from 1 to 7 $mol.m^{-2}.d^{-1}$ and are higher at the Ponta Furada site than in the Caldeirinhas, both in winter and in summer. The duration of non-null PAR signal is shorter in the Caldeirinhas habitat as compared to the Ponta Furada site (from 20 minutes in summer up to 2 hours in winter). These differences are due to the high crater slopes surrounding the Caldeirinhas seabed habitat.

- Coastal waters in the south of Faial can be considered as an unperturbed oligotrophic environment with concentration ranges of 0.12 – 2.81 µM/l for nitrates, 0.13 – 2.23 µM/l for ammonium and 0.03 - 0.17 µM/l for phosphates. In winter, no significant differences in nutrient concentration could be detected between both sites. A summer nutrient depletion was demonstrated and lead to lower nutrient concentrations in the site of Ponta Furada when compared to Caldeirinhas, with significant differences between sites for nitrate and phosphate. Considering each site separately, the summer nutrient depletion is significant for the nitrates and phosphates in Ponta Furada, whereas in the Caldeirinhas they are maintained at relatively high concentrations in summer but with significant (summer) depletion in ammonium. These differences are explained by the more important influence of below thermocline nitrate/phosphate rich waters in the site of Caldeirinhas, and possibly by a more important exposition of the site of Ponta Furada to diffused natural runoff and sewage discharges.

- The average salinity observed in the Caldeirinhas was of 35.9 with frequent intense salinity drop events observed in conjunction with important rain events.

These descriptions of benthic environmental forcing dynamics are produced in support to the interpretation of *Codium elisabethae* population dynamic studied in SAC Monte da Guia, but also foreseeing a further detailed multivariate analysis of links between the present habitat environmental dynamics, the macroalga population dynamics observed by imagery (Chapter 7) and its autecology (Chapter 6).

The present benthic environmental forcing description constitutes also the core of an interesting database, ideally to compile with all other benthic data acquired since then as in the future in order to produce a reliable baseline of benthic seasonal climatology. This will certainly benefit to sustain any future ecological studies focusing on the benthic coastal ecosystems of the Azorean islands, and of the Monte da Guia SAC in particular.

5.5 References

Bashmachnikov, I., and Martins, H., 2006. Upper layer water structure and dynamics in the region of Triangle (Faial, Pico, S.Jorge) from the CTD and moorings data, obtained during ORPAM cruises. Internal report, Oceanography section, DOP. May 2006, 20 pp.

Leliaert, F. and Coppejans, E., 2004. Seagrasses and seaweeds. Standard survey methods for key habitats and key species in the Red Sea and Gulf of Aden. PERSGA Technical Series, 10: 101-124.

Pauly K. and De Clerck O., 2010. GIS-based environmental analysis, remote sensing and niche modelling of seaweed communities, In: Israel A., Einav R. & Seckbach J. (eds.) Seaweeds and their Role in Globally Changing Environments, Springer, 480 pp.
doi: 10.1007/978-90-481-8569-6.

Tempera, F., 2009. Benthic habitats of the extended Faial Island shelf and their relationship to geologic, oceanographic and infralittoral biologic features. PhD thesis, 23-06-09, University of St. Andrews, UK., 341 pp.
http://hdl.handle.net/10023/726

Youssef, W.B.H., 2005. Caractérisation *in situ* de l'environnement physique des habitats benthiques occupés par *Codium elisabethae* au sein de sites d'études particuliers (Faial, Açores). Mémoire de D.E.A en Océanologie. Université de Liège, Liège. 94 pp.

6 - *Codium elisabethae in situ* and laboratory studies

Table of contents

6.1 Introduction and objectives

The general objective is to describe, for the *Codium elisabethae* populations south of Faial Island, the macroalga physiognomy, density of young recruits, reproduction dynamics and nutrient concentration ratio of the water isolated by the lumen. More precisely, this works aim at identifying differences between individuals of populations occupying two distinct habitats and detecting potential seasonal evolutions.

For both study sites, numerous counting, measurements, sampling and subsequent laboratory analysis were carried out all along the study based on a total of 200 individuals collected during the field campaigns of the summer 2003, winter 2004 and summer 2004. These samples were devoted to the following objectives.

The first objective is to produce a complete description of the morphology, ecology and reproductive life cycle of the macroalga through the following observations: horizontal diameter, height, thickness of the thallus, external volume by displacement, internal volume (liquid of the lumen), biomass of the thallus, epiphytic biomass, nutrient concentration in the internal liquid (nitrate, phosphate, ammonium), density of gametangia, number and diameter of the propagules per parent plant.

The second objective is to search and understand potential relations between some of these parameters and the mean horizontal diameter, and particularly to provide the possibility of an indirect estimation of the spatially integrated values of biomass from the population size structure obtained by image analysis.

A third objective of these samplings are the preservation for later complementary studies, as demonstrated by the phylogenetic positioning of *Codium elisabethae* within the *Codium* genus (Leliaert, personal communication, 2007) and by the identification of several endophytic cyanobacteria leaving entangled within the inner part of the thallus, in contact with the lumen water.

6.2 Material and methods

6.2.1 *In situ* counting's and measurements

Along the study, *in situ* counting's and measurement were performed to provide a basis of comparison with the results obtained with imagery, and to produce a closer estimation of younger individual densities and dynamics. 42 repetitions of this counting were performed, 16 in the Caldeirinhas, 26 in the site of Ponta Furada. The methodology consisted of placing a 1 by 1 meter thin metallic quadrat on the bedrock, its corners being positioned over nails fixed in the rock, within the study sites devoted to visible imagery experiments. A close photo of the quadrat in place was taken for good identification within the larger image mosaics. The measurement of the diameter of all individuals present was done with a caliper (mm precision) and with a 0.5 cm graduated meter band for individuals larger than 22 cm. Subdivision of the zone was made easier by the use of a gridded smaller metallic quadrat, and measures were communicated to a second diver for hand recording (Fig. 1). For ovoid shaped thallus, both long and small horizontal diameters were recorded for estimation of a "mean

horizontal diameter" as seen from above. Limited by to scuba diving imperatives (depths of 20 to 23 meters), the time consuming counting was possible for a maximum of three square meters per dive in Ponta Furada, and to only one square meter for the more densely populated site of the Caldeirinhas.

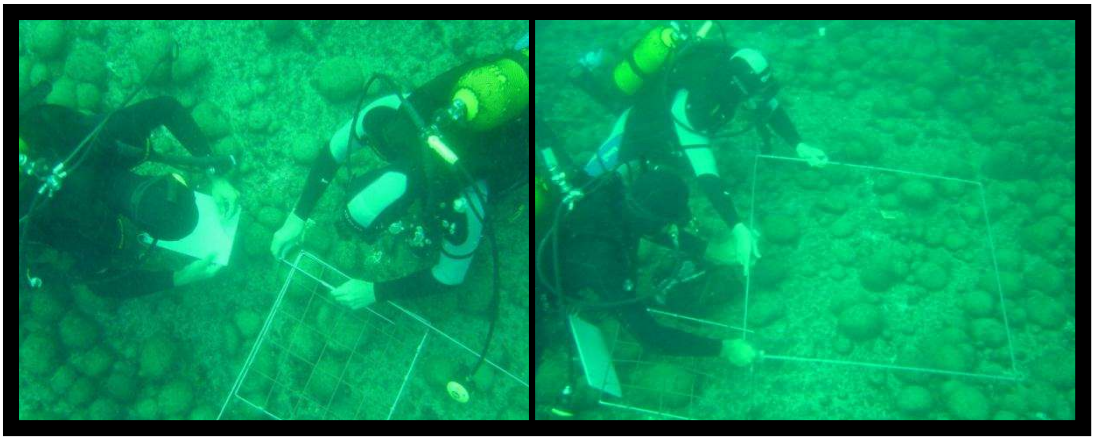


Figure 1 - *In situ* measurement of population structure, site of Caldeirinhas

6.2.2 *Codium elisabethae* sampling

About 200 *Codium elisabethae* have been collected at the proximity of both study sites in August 2003, February, July, August, November 2004, August and November 2005. Each collecting campaign targeted to collect a minimum of 10 individuals, chosen to represent as evenly as possible the class size ranging from 3 cm to 25 cm, and collected at least 50 meters away from the study sites in order to reduce possible sampling influence on population dynamic studies. These samples were brought directly to the laboratory after collection and used for the various laboratory studies mentioned hereafter.

6.2.3 Morphology

Height, maximal and minimal diameters were measured as described for *in situ* measures (6.2.1). After eliminating all propagules, sediments and epiphytes, the displaced volume of the alga was measured by immersion in a graduated container partially filled with water. Internal volume was measured by collecting lumen water by drainage into a graduated container, and then total fresh biomass was measured on precision balance. Once cut out, thickness of thallus was measured with a caliper. At that stage, small thallus samples (2 by 2 cm) were cut out from the thallus of 140 individuals (Table 1) and conditioned frozen in prevision of various later studies (sexual reproduction, genetics, internal cyanobacteria). Remaining part of the thallus was weighted on precision balance before and after drying in oven (standard procedure 12h at 50°C) so as to define the dry weight to fresh weight biomass ratio.

6.2.4 Reproduction

6.2.4.1 Sexual reproduction

A counting of the proportion of utricles showing gametangia was carried out on samples extracted from 140 *Codium elisabethae* collected for both sites during 4 periods (February 2004, July-August 2004, December 2004 and March 2004) (Table 1). For numerous of these samples, pieces of the algae were cut out from top, lateral and lower parts of the thallus.

Sampling period	February 2004		July-August 2004		December 2004		March 2005	
Site	Cald.	P.F.	Cald.	P.F.	Cald.	P.F.	Cald.	P.F.
Number of samples	21	19	22	18	20	20	10	10

Table 1: Number of *Codium elisabethae* exploited for studies on sexual reproduction

A method was established for the consistent evaluation of total density and proportion of full gametangia within these samples (work supervised within Desclin, 2005). Counting's and observations were made with a binocular at (10x/23 x 5) magnifying factor with adapted camera frame grabbing, cumulating all gametangia, without distinction of gamete type (male or female). In summary, after checking the effect of various parameters (method of de-freezing the samples and use of sweet or salty water on utricle turgescence, surface of thallus and number of utricles to observe to produce a stabilized answer concerning density of utricles showing gametangia) we could select an optimal approach and demonstrate the importance of focusing the measure on a specific part of the thallus as significant variability of the density of gametangia occurs between the basis, lateral part and the tip of these *Codium* (Annex 10.2). For 18 individuals, the gametangia counting were repeated on the same sample and confirmed the good reproducibility of the observations for most of the samples.

With this approach, we investigate the variations of sexual reproduction intensity by following the density of utricles carrying one or more gametangia and by the proportion of them still holding gametes. These data are aggregated and compared according to the site, parent plant size, and season.

6.2.4.2 Vegetative reproduction

For a total of 185 individuals collected (104 individuals from the Caldeirinhas and 81 from Ponta Furada), the total number and weight of propagules present on each parent plant have been recorded, and their size recorded with a caliper. Thus the effects of site, parent plant size, and season on the dynamic of vegetative reproduction are also analyzed.

6.2.5 Nutrient concentration within the lumen of *Codium elisabethae*

In order to detect the nutrients concentrations (total nitrates NO_3^- and nitrites NO_2^-; phosphates PO_4^{3-} and ammonium NH_4^+) within the lumen water of the studied populations, a total of 160 water samples were collected *in situ* (40 in winter and 39 in summer within the Ponta Furada population, and 38 in winter and 42 in summer within the Caldeirinhas population).

Water sample were collected *in situ* with syringe equipped with a needle. Samples were then treated as explained in Chapter 5, section 5.2.2 for surrounding seawater.

6.2.6 Epiphytism on Codium elisabethae

For the 80 individuals collected in February and July 2004 (table 1), the total epiphyte cleaned out from the thallus was weighted as fresh biomass.

6.3 Results and discussions

6.3.1 *In situ* countings and measurements

6.3.1.1 Initial observations on population structures

In August 2003, two repetitions of this counting method revealed densities of 84 and 133 individuals per square meter in the Caldeirinhas, with mean diameter respectively of 7.4 and 4.8 cm. In the site of Ponta Furada, three repetitions revealed densities of 8, 14 and 17 individuals per square meter, with mean diameter respectively of 12.6, 11.8 and 11.6 cm. The difference of mean diameter between both sites reflects the older population structure in Ponta Furada. Although these counting are devoted to validation of imaging results, they showed that larger surface must be covered by *in situ* counting to provide representative values of densities in the delimited study sites. Yet, these first results showed clearly the general differences of populations between the two sites: an average density of 110 individuals.m^{-2} was measured *in situ* in the Caldeirinhas site, against only 13 individuals.m-2 in the site of Ponta Furada. Structures of populations were also well distinct, with a more continuous size distribution in Caldeirinhas stands, although large individuals (above 18 cm diameter) were more frequent in Ponta Furada (Fig. 2), and reached larger maximal size than in the Caldeirinhas (32 cm against 24 cm). These values observed in the study sites of Faial are notably larger than the maximum diameter reported by earlier population studies achieved in the western Azorean island of São Miguel (Neto, 1997 and 2000). Generally a larger mean horizontal diameter is observed in Ponta Furada, reflecting the older population structure.

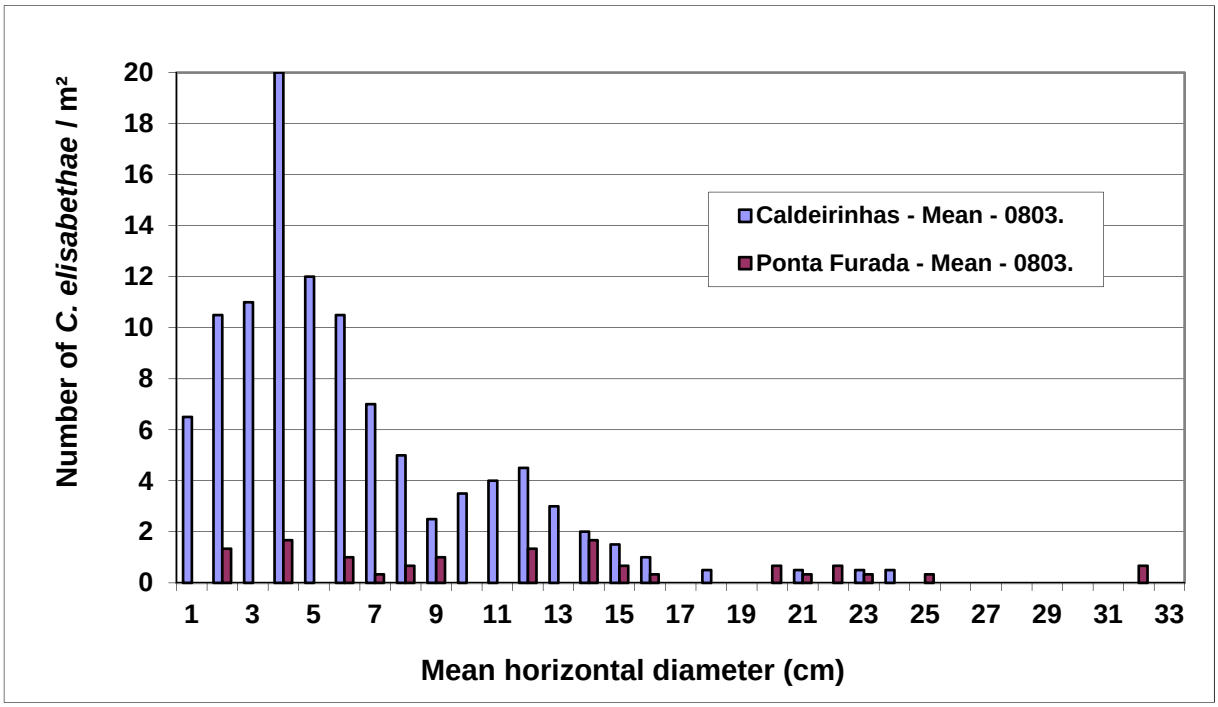


Figure 2 – Mean size histogram measured in august 2003 in Caldeirinhas and Ponta Furada sites by *in situ* counting method.

6.3.1.2 Spatio-temporal variability of density

Populations were re-evaluated in the exact same quadrats in February 2004 (Fig. 3), showing important reduction of average density in the site of the Caldeirinhas (from 110 to 61 ind./m²; i.e. - 44%), and almost steady state in Ponta Furada (13 to 12 ind./m²; i.e. –8%). This difference can be attributed to the higher mortality (* disappearing from the zone, fate to be further investigated, most probably more intense grazing by *Sarpa salpa* dense schools) of younger individuals which were more represented in the summer in Caldeirinhas. This stronger population drop can also be linked with a notion of saturation and intra-specific competition for space in the Caldeirinhas, growing individuals exerting increasing pressure on the surrounding ones, turning them more sensitive to strong hydrodynamic events.
Mean density of young recruits produced by sexual reproduction (diameter < 2 cm) was clearly higher in the site of the Caldeirinhas (order of 20 ind./m²) than in Ponta Furada (order of 1/m²), for both periods. The low density of recruitment remained almost constant in Ponta Furada, whereas more intense dynamics was observed in Caldeirinhas: while one quadrate showed clear increase of young individual density from summer to winter (9 to 34 ind./m²), another one distant of 5 meters showed opposite trend from 25 ind./m² in the August to 11 ind./m² in February.

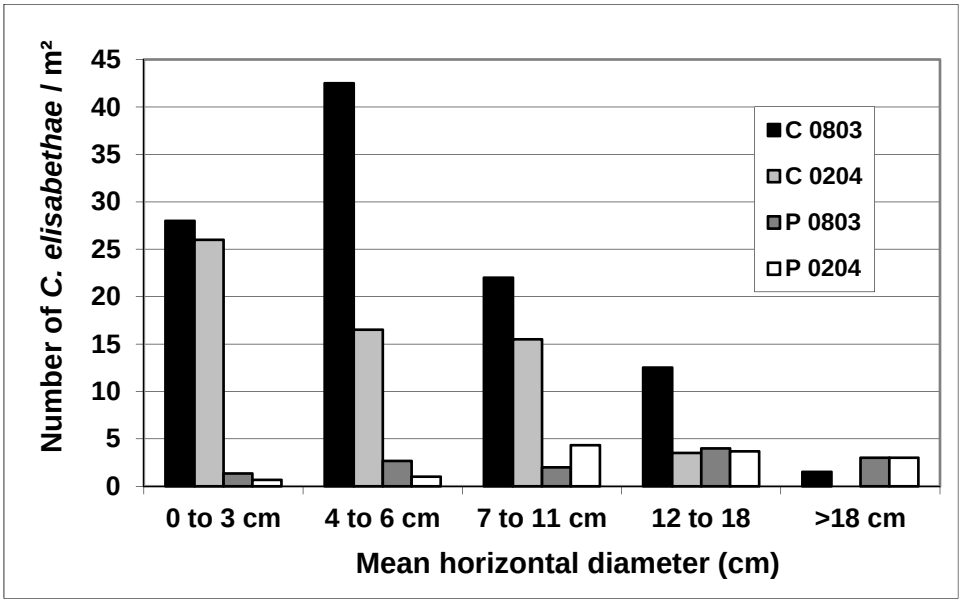


Figure 3 – Synthesis of population structure evolution seen from *in situ* counting between August 2003 and February 2004 for both Caldeirinhas (C) and Ponta Furada (P) sites.

The summer 2005 mission allowed and extensive *in situ* measurement of population structure within 6 m². Four squares were positioned at the 4 corners of each study site, and two other at the middle of the long side. Nails in the rock identified each position. These counting confirm the general differences of population structures previously suggested (Fig. 4): higher density and more continuous size distribution for Caldeirinhas population, whereas bigger individuals (diameter > 15 cm) are more frequent in the site of Ponta Furada, and reach larger maximal size than in the Caldeirinhas (21 cm against 16 cm).

Reproduction dynamics and temporal variability of density seems more important for the site of the Caldeirinhas. Per square meter, an average of 40 individuals smaller than 5 cm were counted over a total 64 individuals in the Caldeirinhas, whereas only 3 individuals smaller than 5 cm were observed over a total de 8 individuals in Ponta Furada.

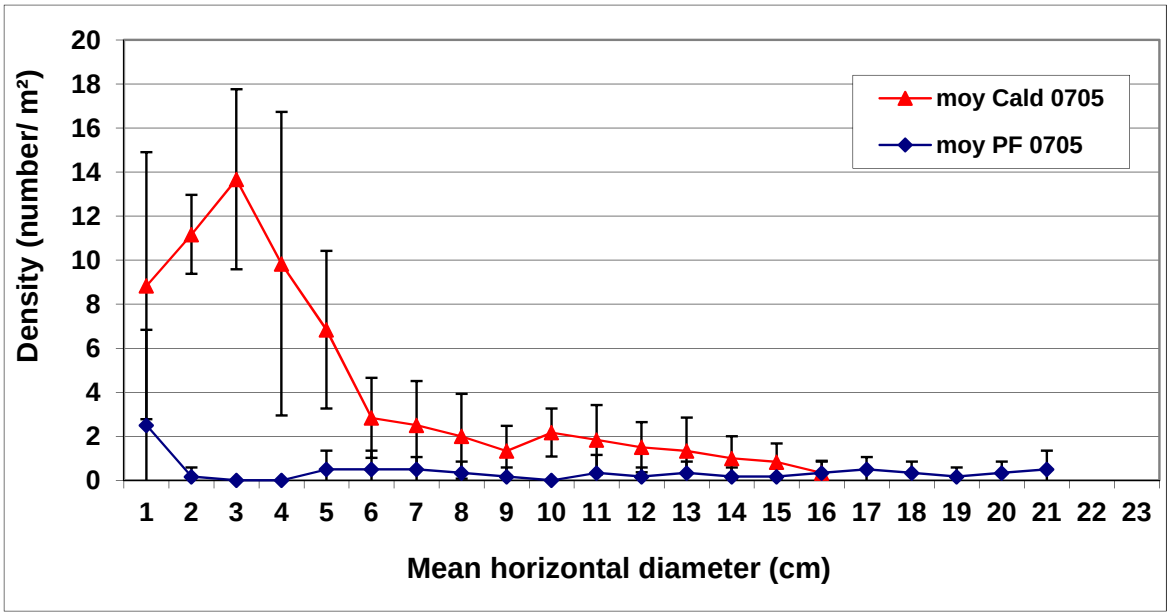


Figure 4 – Size histogram obtained from *in situ* counting on 6m² per site in July 2005.

These *in situ* counting revealed an important spatial variability of structures and densities at small spatial scale within each of the sites, both during summer and winter periods. The data suggests potential differences in recruitment dynamics between the sites and that general strong small scale patchiness processes affects population structure. With the *in situ* counting methodology described, this patchiness can be quantified only at the scale of the quadrat surface, each quadrat being the unit of measurement, which prevent analysis of sub quadrat organisation and is limited in spatial repetitions because being highly time consuming.

21 *in situ* countings were exploited to build contingency tables for comparison of both *in situ* and imagery counting methods.

6.3.2 Morphology

According to the literature, it is classically admitted that the only distinction allowing differentiating *Codium elisabethae* from *Codium bursa* is the presence of an acuminate mucron at the tip of the utricles for the species *Codium elisabethae*. On one of the sample of *Codium elisabethae* brought back to the laboratory, a case of double tip could be observed with microscope on one utricle (Fig. 5). Until diameters of 17 cm, the increase in thallus height with mean horizontal diameter can be roughly considered linear (Fig. 6), but with a ratio of about 0.72, reflecting the general flattened shape of the thallus as compared to a sphere.

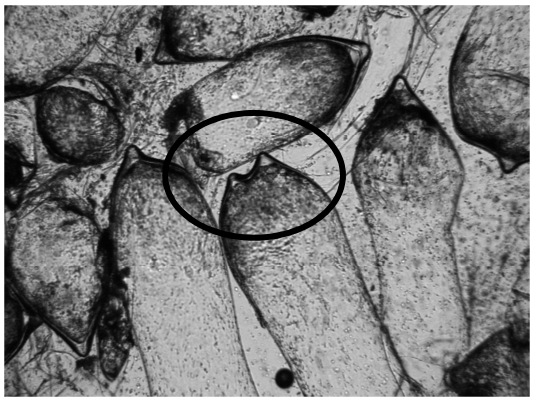


Figure 5 – Double mucron at the tip of a *Codium elisabethae* utricle

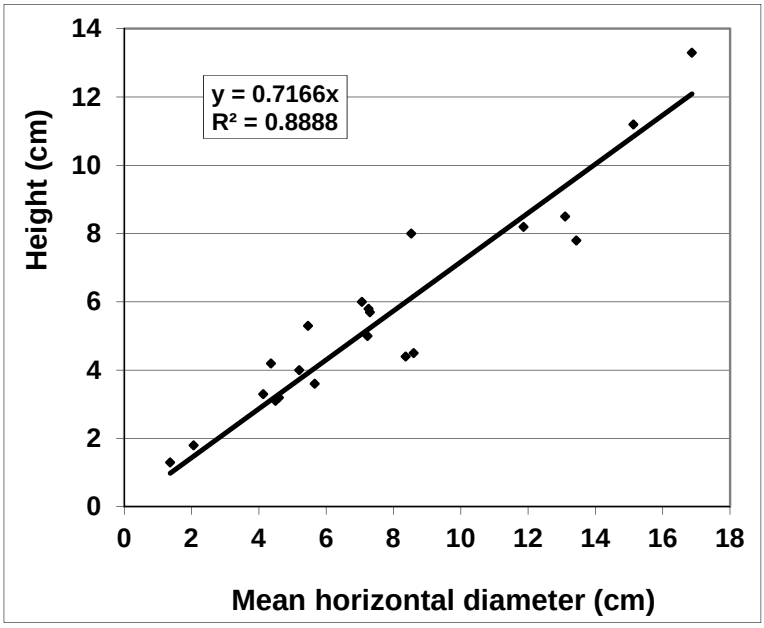


Figure 6– Relation between mean horizontal diameter and height of *C. elisabethae.*

C. elisabethae biomass is correlated to mean horizontal diameter, and this will be exploited later for the estimation the local variations of this algal biomass using individual size estimation derived from image analysis. A power relation with exponent higher than two (at least 3) is logically a satisfying model for this as the alga sphere thickness rises also during growth. An optimal correlation (r^2=0.932) was reached with a negative factor weighting a 4^{th} degree exponent term. This does reflect the fact that the alga shape gets progressively flattened at later life stages (Fig. 7).

The average ratio measured in laboratory between dry (g. dry weight) to fresh biomass (g. fresh weight) of *Codium elisabethae* thallus is of 0.068, and was used for conversions in later processing. From an initially spherical shape, the flattening evolution is more pronounced for the Ponta Furada population, as measured by the evolution of global and internal volumes versus mean horizontal diameter (Fig. 8).

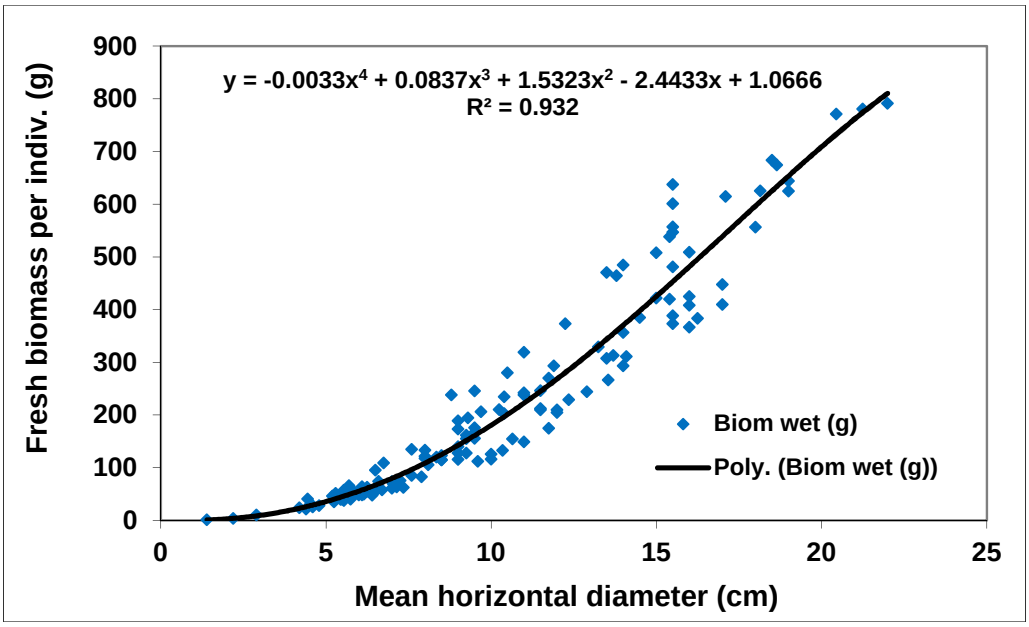


Figure 7 – Evolution of *Codium elisabethae* fresh biomass versus mean horizontal diameter and fitted polynomial used for biomass estimates from imagery.

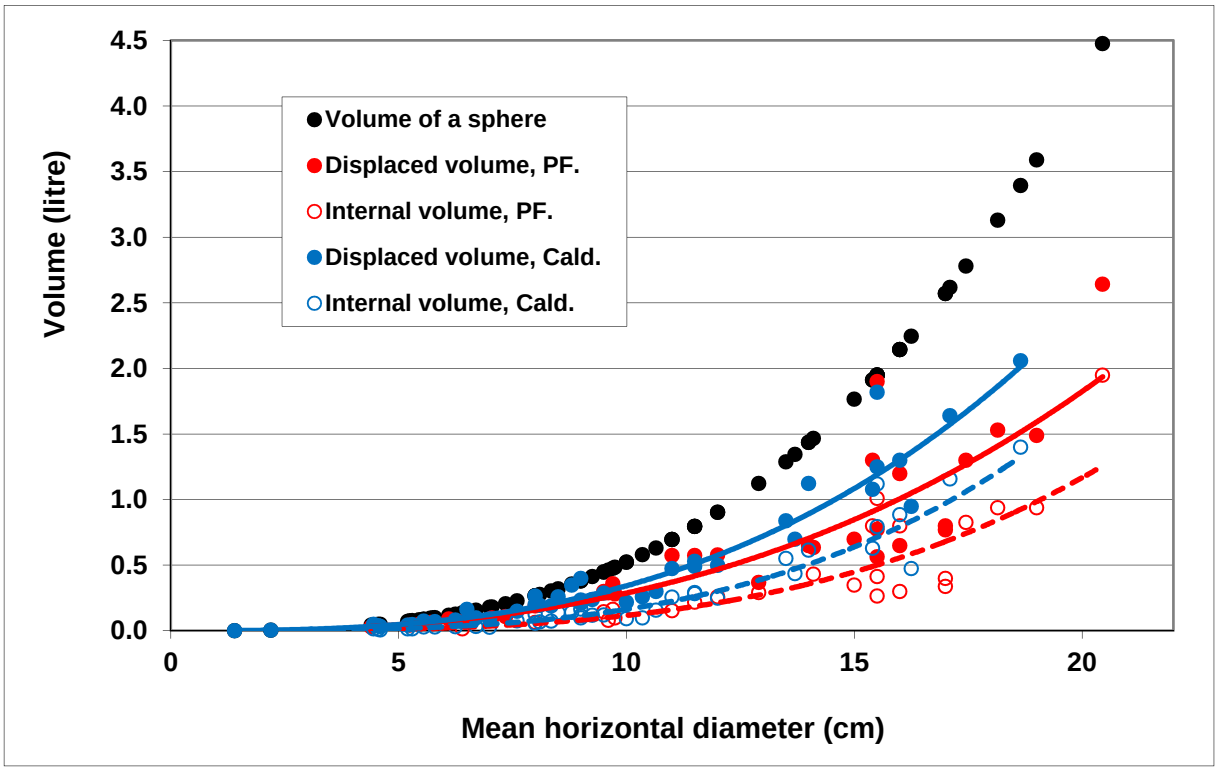


Figure 8 – Total displaced and internal volume of *Codium elisabethae* versus mean horizontal diameter, for each site (blue=Caldeirinhas; red=Ponta Furada; volume of a sphere).

Both species thallus were observed to be attached by rhizoidal filaments, mostly anchoring into crustose *Corralinaceae* (calcareous red algae; as in Faial island, Azores; Calvi Bay, Corsica, France) or even on calcareous reefs formed by *Halimeda tuna* (as in Green Island, La Ciotat, France).

6.3.3 Reproduction

Both sexual and vegetative reproduction strategies were observed for the populations of *Codium elisabethae* encountered around Faial Island.

6.3.3.1 Sexual reproduction

In august 2003, a first gametangium was observed in summer (Fig. 9), on utricle of an individual from Caldeirinhas. Such an observation in the summer was particularly interesting, even yet without precision on gamete content or type, as all previous studies were indicating a reproduction season spanning only over the fall and winter. This raised the question of the specific dynamic of sexual reproduction of *Codium elisabethae* populations in the Azores and in our references sites of SAC of Monte da Guia in particular.

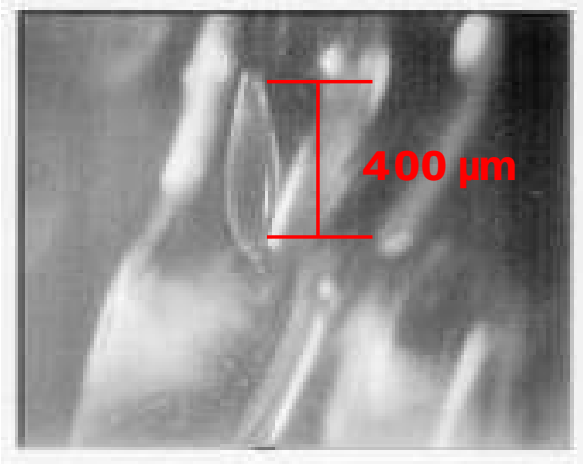


Figure 9 – Microscopy photography of gametangia observed in August 2003 on a *Codium elisabethae* from the Caldeirinhas site.

The gametangia counting results are synthesized by a mean value and standard deviation per site and period (Fig. 10), representing the general investment of the algae in sexual reproduction.

The density of utricles with gametangia was shown to be significantly higher in December than in February and summer periods, for each site (Anova test 1 factor, Desclin, 2005).

In summer, the reproduction is less intense than in winter, but it is not absent, in contrast with earlier statements made for the *Codium elisabethae* populations of the Azorean island of São-Miguel island (Neto, 1997). This information is an important confirmation of the active summer reproduction which was already suggested by our *in situ* observations of numerous young recruits during summer 2003.

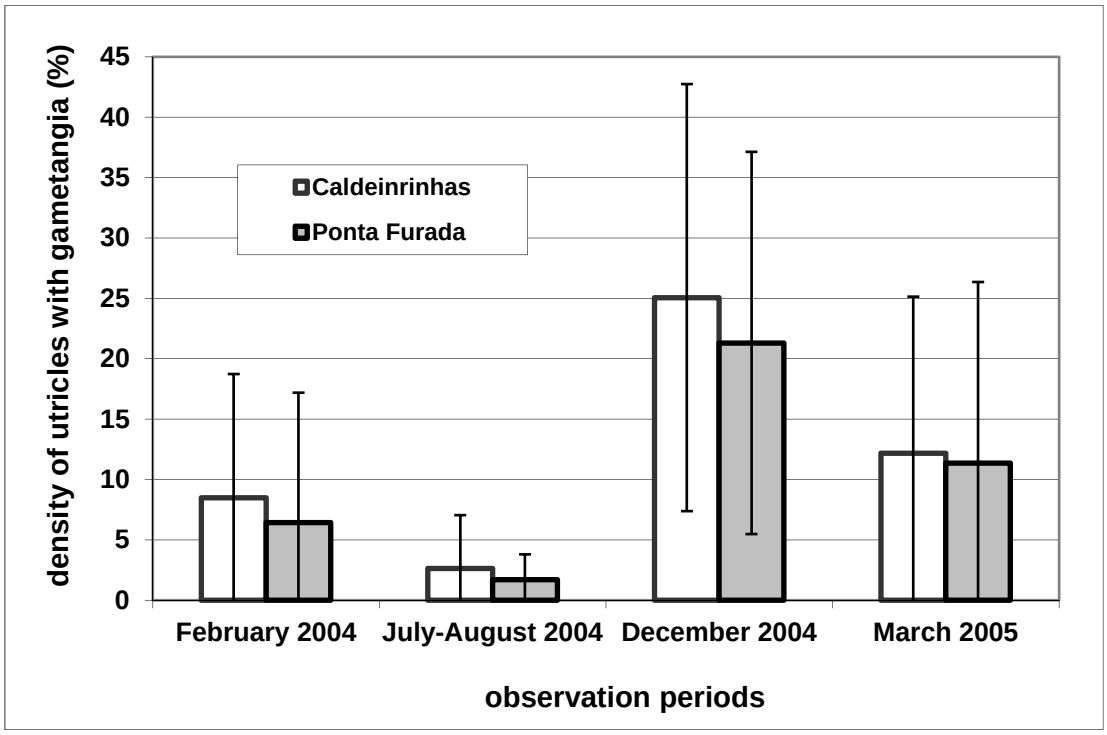


Figure 10 – Mean density of utricles with gametangia per site and observation period

The density of utricles carrying gametangia tends to be higher for the population of the Caldeirinhas for each period (Fig. 10), but no significant statistical differences could be demonstrated with the data gathered, whether considered separately by period or all aggregated.

Using Principal Component Analysis, Desclin (2005) identified the density of gametangia carrying utricles to be correlated with the large horizontal diameter and small horizontal diameter of the algae, according to period and site studied. Both horizontal dimensions of the algae carrying information, it was chosen here to analyse data as function of the mean horizontal diameter of the algae. When looking at the data of each site and each season taken individually (Figs. 11 and 12), a trend to positive correlation can be observed between the density of utricles carrying gametangia and the mean horizontal diameter of the sampled algae. When aggregating these data for both sites, the general positive correlation between density of utricles with gametangia and mean horizontal diameter is shown to be statistically significant ($p<0.05$) for all periods tested, even if the linearity of the relation is rather low (low r^2). Maximum densities of gametangia are reached for mean horizontal diameter ranging from 10 to 18 cm. Although the low amount of data concerning the largest individuals, it even appears that a trend of reduction of the density of utricles with gametangia appears after reaching a maximum, above a diameter of 18 cm in February 2004; of 15.5 cm in July-August 2004, while the highest values are reached for individuals of about 10 cm in December 2004, followed by a plateau (high values between 30 and 50 %), maintained till 18.5 cm.

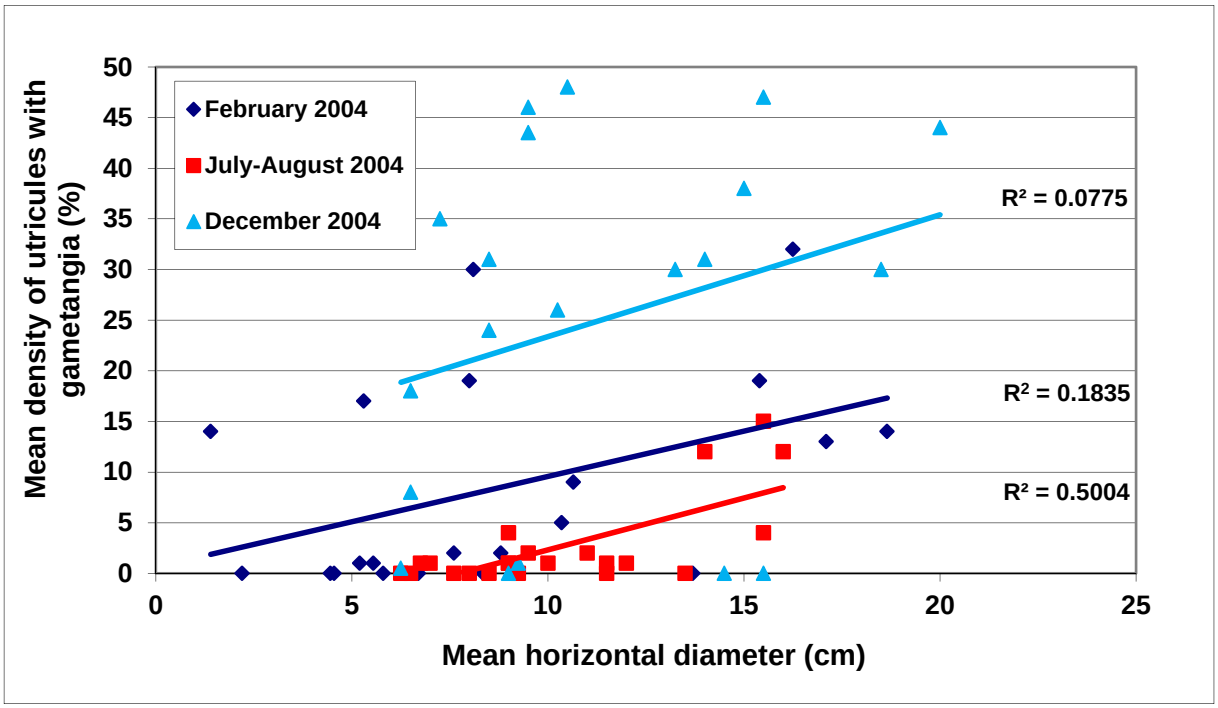


Figure 11 – Distribution of mean density of utricles carrying gametangia according to the algae size, site of Caldeirinhas.

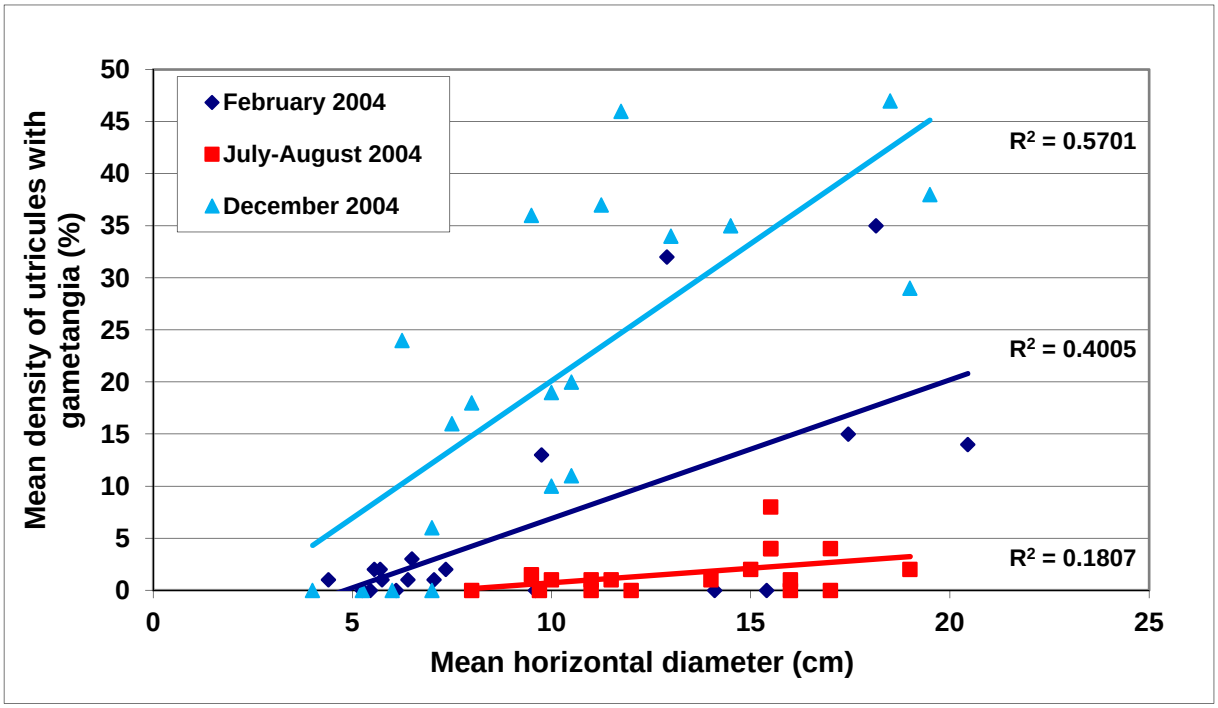


Figure 12 – Distribution of mean density of utricles carrying gametangia according to the algae size, site of Ponta Furada.

If in February the maximal values are of the same order for both sites (32 à 35 %), it appears nevertheless that in the site of Ponta Furada, the reproduction is very low for individuals below 10 cm diameter, which is not the case for the population of the Caldeirinhas.

Generally, we observe an early onset of sexual reproduction, which is also more intense for the population of the Caldeirinhas, comparatively to the one of Ponta Furada.

In order to complete the information carried by the density of utricles carrying gametangia, the proportion of the counted gametangia that are still carrying gametes are illustrated in figure 13. The proportion of full gametangia observed is also maxima in December for both sites, and the reproduction is confirmed to be still active in summer.

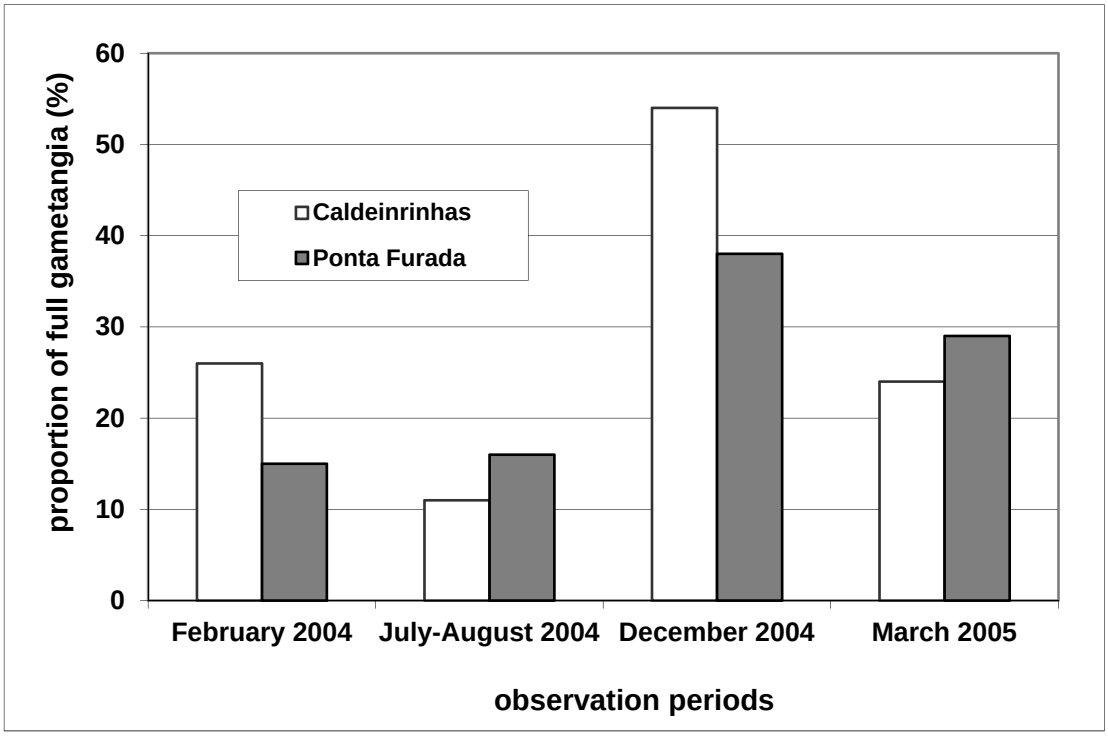


Figure 13 – Proportion of gametangia carrying gametes, full per site and observation period.

6.3.3.2 Vegetative reproduction

Most of the propagules were observed growing on the lateral parts of the algae thallus (Fig. 14).

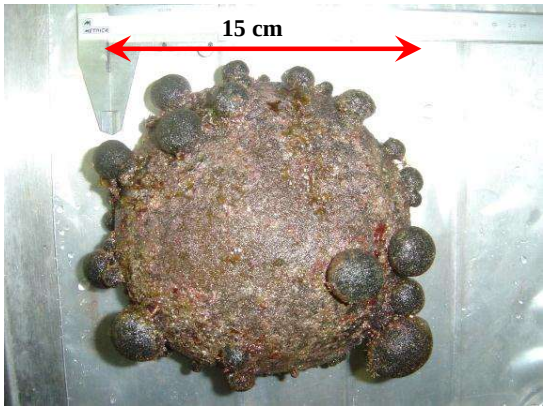


Figure 14 – *Codium elisabethae* in intense vegetative reproduction process (diam. 15 cm)

Propagules are attached by thick cellular filaments crossing the utricles wall of the parent alga and being entangled further into the underneath matrix of cellular siphons. These fixations are relatively resistant as only strong traction can extract them as far as one centimeter before breaking. The morphology of the lower part of the propagule fixation presents an interesting « rooted » shape divisions (Fig. 15).

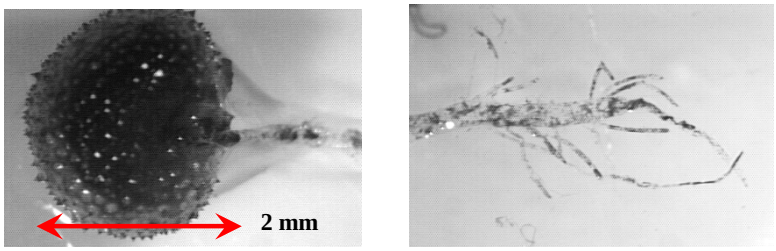


Figure 15 – Detail of a small propagule and fixation filament (left), with its rooted shape lower extremity (right)

The total number of propagules counted per alga (considered hereafter as the intensity of vegetative reproduction) is plotted versus the mean horizontal diameter of the parent alga in figures 16 and 17. Data are grouped into summer and winter subsets for illustration, but for both sites, no significant statistical effect of the collecting periods could be detected (Test U Mann and Whitney: $p < 0.4$ for Ponta Furada, $p = 0.42$ for Caldeirinhas; Desclin, 2005).

Overall, the vegetative reproduction is much more intense for the population of the Caldeirinhas with frequent parent plant carrying up to 10 to 60 propagules (and one record reaching 167 propagules on a single plant), comparatively to the vegetative reproduction observed in Ponta Furada, with a maxima of 8 propagules per individual. For each collecting period tested, the difference between the two sites is significant (Test U Mann and Whitney: $p = 0.05$; Desclin, 2005).

This mode of reproduction was observed to start only for individuals reaching a mean horizontal diameter of 6 cm. For the Caldeirinhas population, a first reproduction phase is observed for some of the individuals between 6 and 9 cm, and is followed by an almost absent propagule production during the growth period from 9.5 till 11.5 cm. Then a second vegetative reproduction phase, more intense, is observed for individuals above 11.5 cm, reaching a maximum for a 16 cm plant. Above this diameter, most individuals do show on-going vegetative reproduction. For the Ponta Furada population, the first reproduction phase is observed for some of the individuals between 6 and 11 cm, and is followed by an almost absent propagule production during the growth period from 11 till 15 cm. Then a second vegetative reproduction phase is observed for individuals above 15 cm, yet for about only half of them.
U Mann and Whitney tests performed for each site and for three periods (February 2004, summer 2004, December 2004) and confirmed the significant effect of the diameter of parent alga on the number of propagules carried (Desclin, 2005).

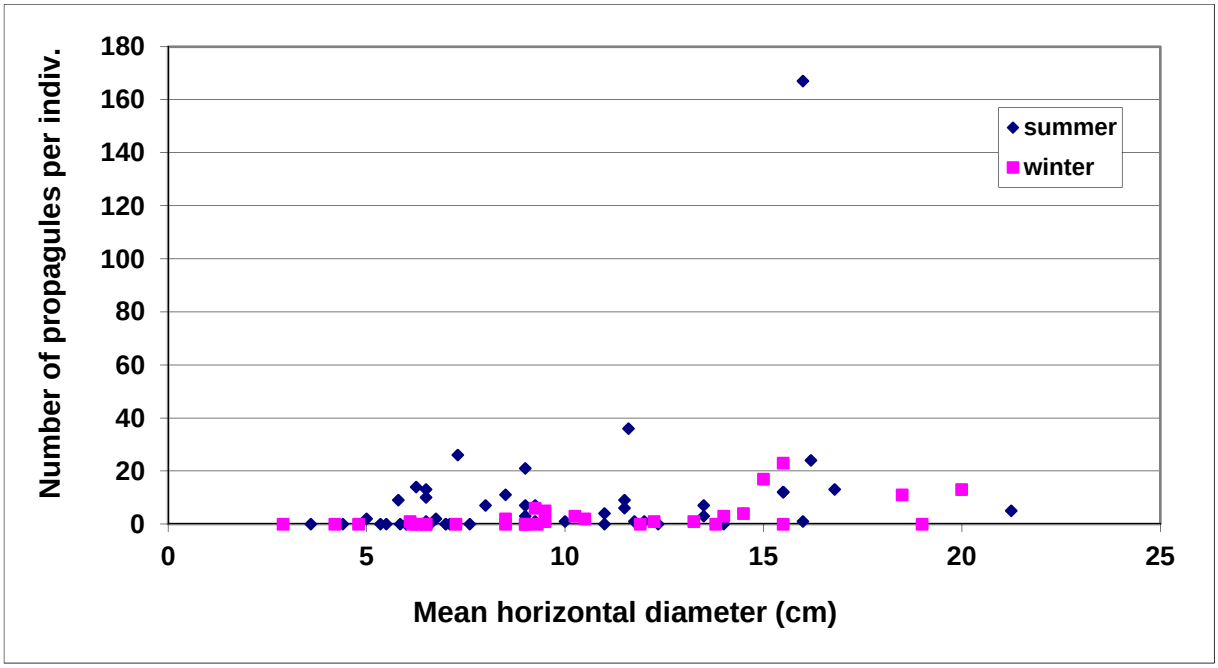


Figure 16 – Number of propagules per parent plant, site of the Caldeirinhas.

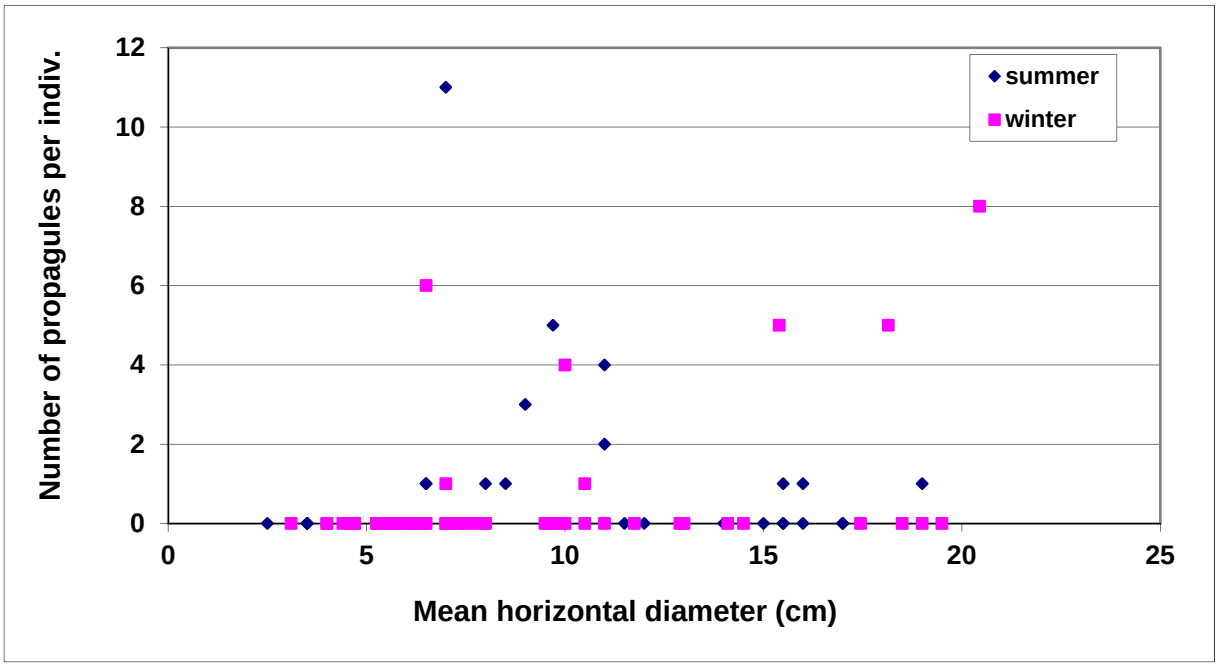


Figure 17 – Number of propagules per parent plant, site of Ponta Furada.

The production of new young propagules (1-3 mm diameter) is reaching a maximum for individuals of 11.5 to 17 cm in the site of Caldeirinhas, while this maximum is reached for individuals of 10 cm in the site of Ponta Furada. The maximal diameter of the propagules rises with the size and age of the parent alga. The biggest propagules observed still attached to a parent plant reached 4.5 cm on a 15.5 cm alga of the Caldeirinhas, whereas the biggest propagules observed on *Codium elisabethae* from Ponta Furada had a diameter of only 0.5 cm. The final fate of these propagules is still unstudied. Some might stay attached to parent plant as a substrate till decomposition of this last one, or might mostly finish by being detached by hydrodynamic actions and then fixating on the substrate and growing, or being advected away, dying and decomposing. The proportion of these possible pathways remains to be quantified to allow precise population dynamic modelling and understanding.

6.3.4 Nutrient concentration within the lumen of *Codium elisabethae*

Season and site effects on internal nutrients aggregated on all class sizes

Aggregating all data independently of the algae diameter, Fisher statistical test shows that in summer, nutrients are non-significantly different in the internal water of the populations of both sites (p=0.811 for nitrate; p=0.458 for ammonium; p=0.899 for phosphate; Fig. 18). In winter, all nutrients concentrations tend to be superior in the population of Ponta Furada, but the difference is only significant for ammonium (p=0.068 for nitrate; p=0.0047 for ammonium; p=0.622 for phosphate; Fig. 18).

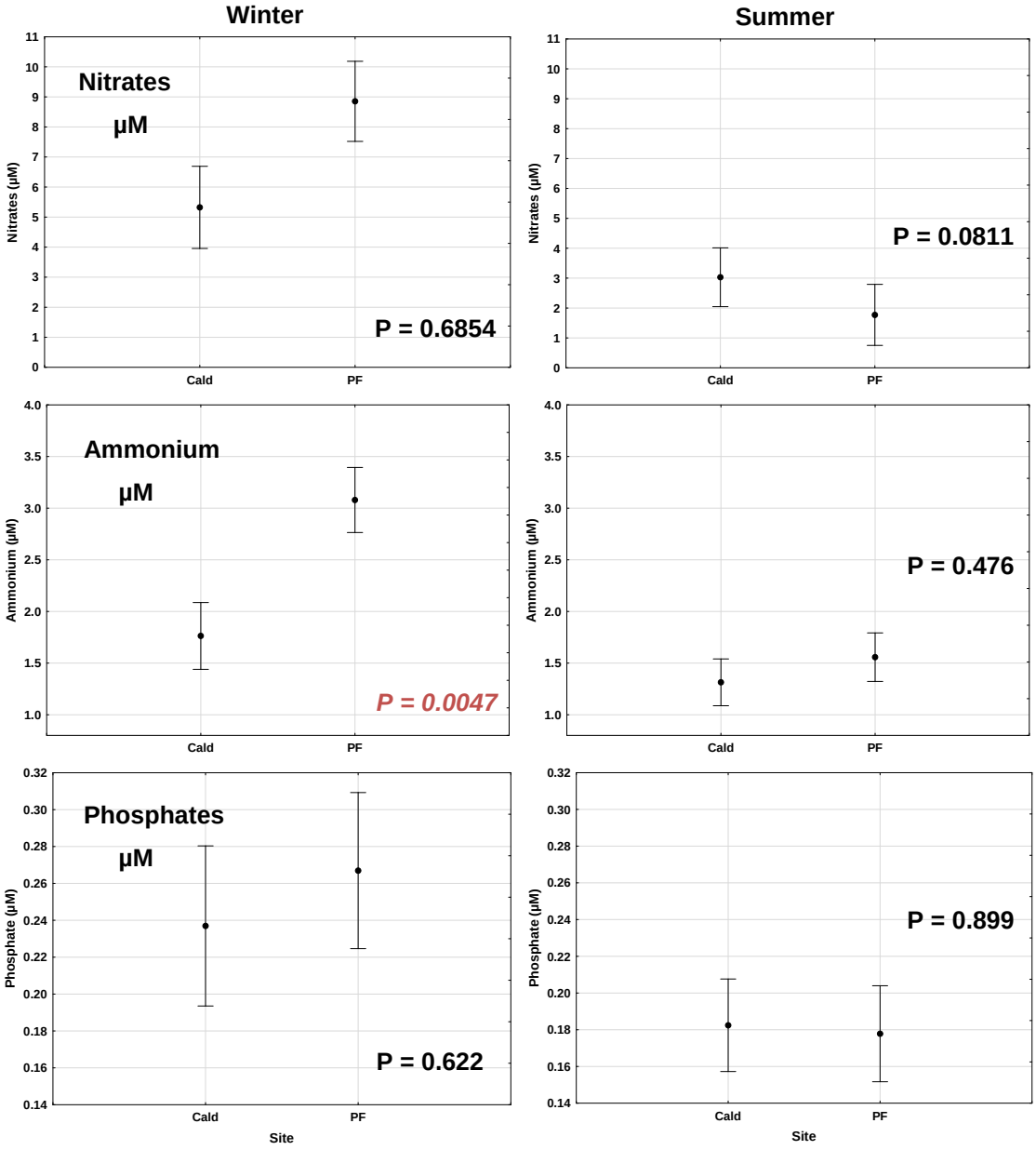


Figure 18 – Comparison of mean nutrient concentrations measured per site in the lumen of *Codium elisabethae*, for both winter (left column) and summer (right column); vertical bars denote +/- standard errors; p=significance of Fisher tests concerning site differences.

Aggregating all data independently of the algae diameter, Fisher tests show that in the site of Ponta Furada, summer concentrations of internal nutrients (nitrate, ammonium and phosphate) are all significantly lower than in winter (Fig. 19; p=0.00007 for nitrate; p=0.00236 for ammonium; p=0.02968 for phosphate). In the site of Caldeirinhas, the summer concentrations are only slightly reduced, and the difference is only barely significant for the nitrate (p=0.04017 for nitrate; p=0.10382 for ammonium; p=0.34338 for phosphate).

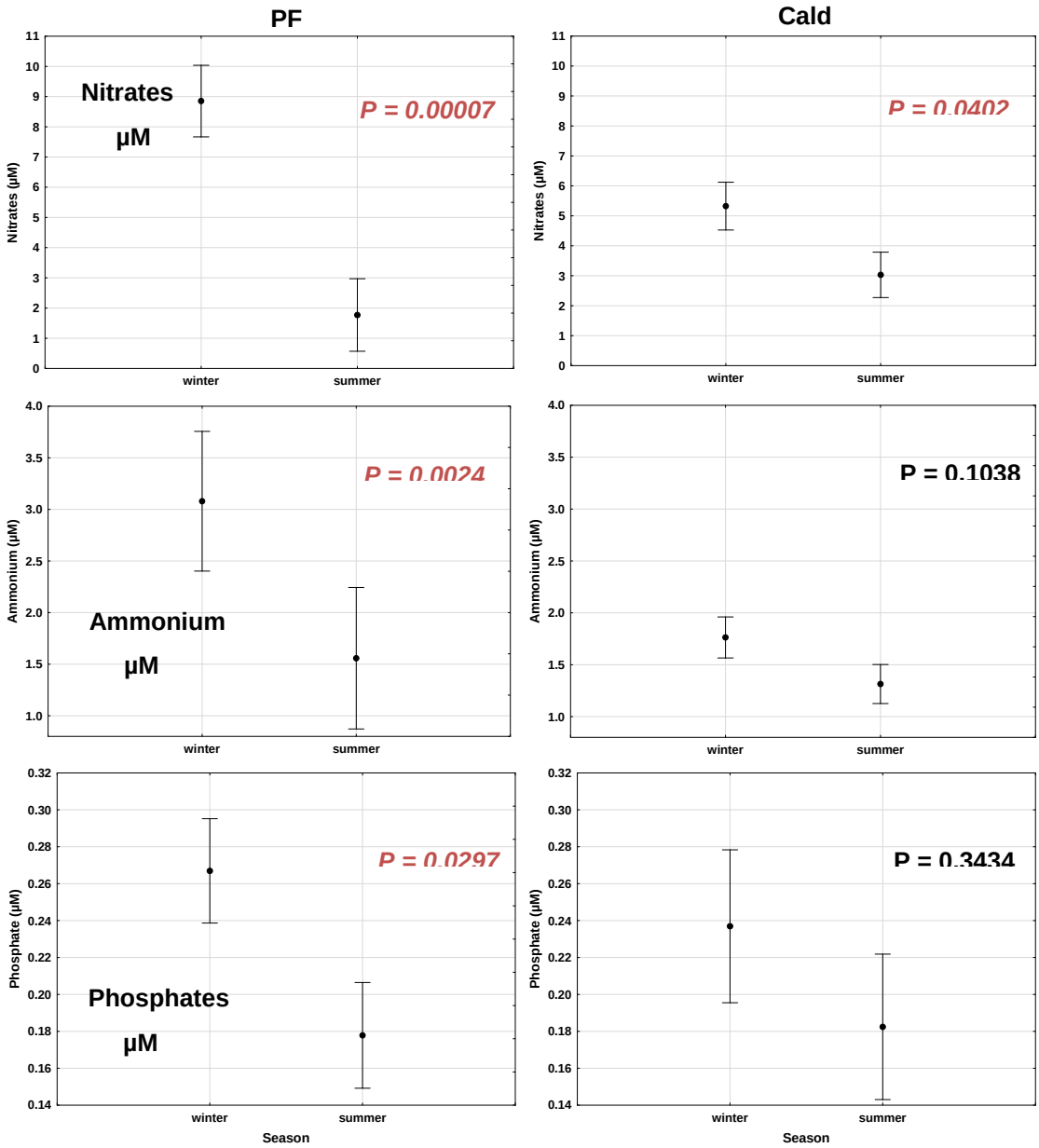


Figure 19 – Comparison of mean nutrient concentrations measured per season in the lumen of *Codium elisabethae*, for both sites (Ponta Furada left column; Caldeirinhas right column); vertical bars denote +/- standard errors; p=significance of Fisher tests concerning seasonal differences.

Class size effect on internal nutrient concentrations, aggregating data independently from seasons and sites.

While analyzing the effect of plant class size on the lumen nutrient concentrations independently from seasons and sites, no significant effect could be detected globally. Nevertheless, for all 3 nutrients evaluated, maximum concentration values are observed for the younger algae (diameters ranging from 6 to 10 cm), whereas a following algal life stage starting at diameters of 11-12 cm is characterized by minimum nutrient concentrations values (Figs. 20, 21 and 22). These observations remain as trends probably due to limited sampling replicates, and would probably deserve some complementary sampling to establish them as significant. From the data collected, an interesting point raised by a detailed look at the lumen nutrient concentrations plotted per class size, per site and per season (Annex 10.3) is that all nutrient concentrations tend to be maximum in February and minimum in July-August in both sites, but in December the concentrations are still remaining low in the Caldeirinhas population, while already starting to raise in the population of Ponta Furada.

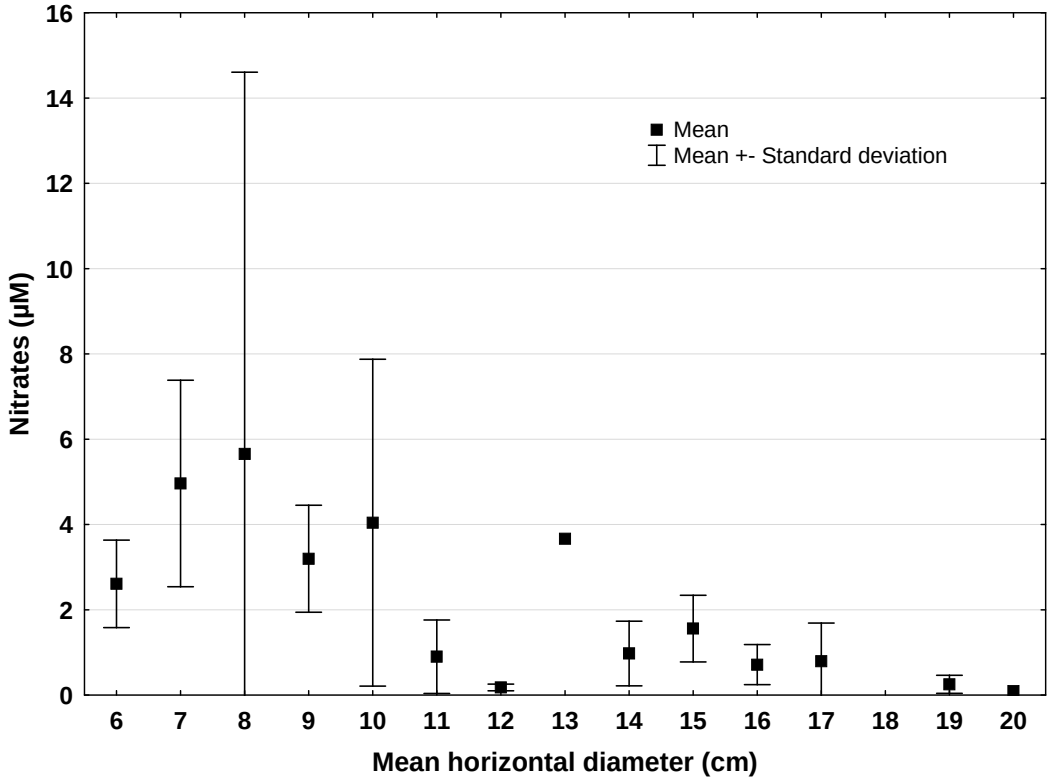


Figure 20 – Nitrate concentrations in the lumen of *Codium elisabethae versus* algae class size, data aggregated over season and sites.

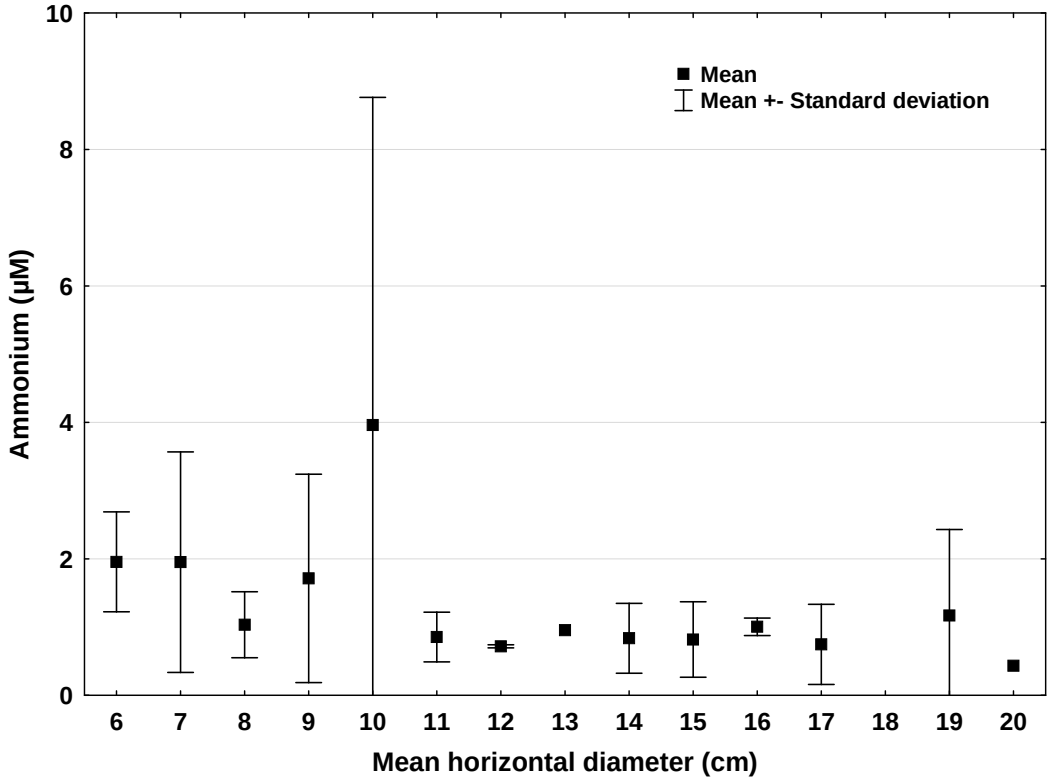


Figure 21 – Ammonium concentrations in the lumen of *Codium elisabethae versus* algae class size, data aggregated over season and sites.

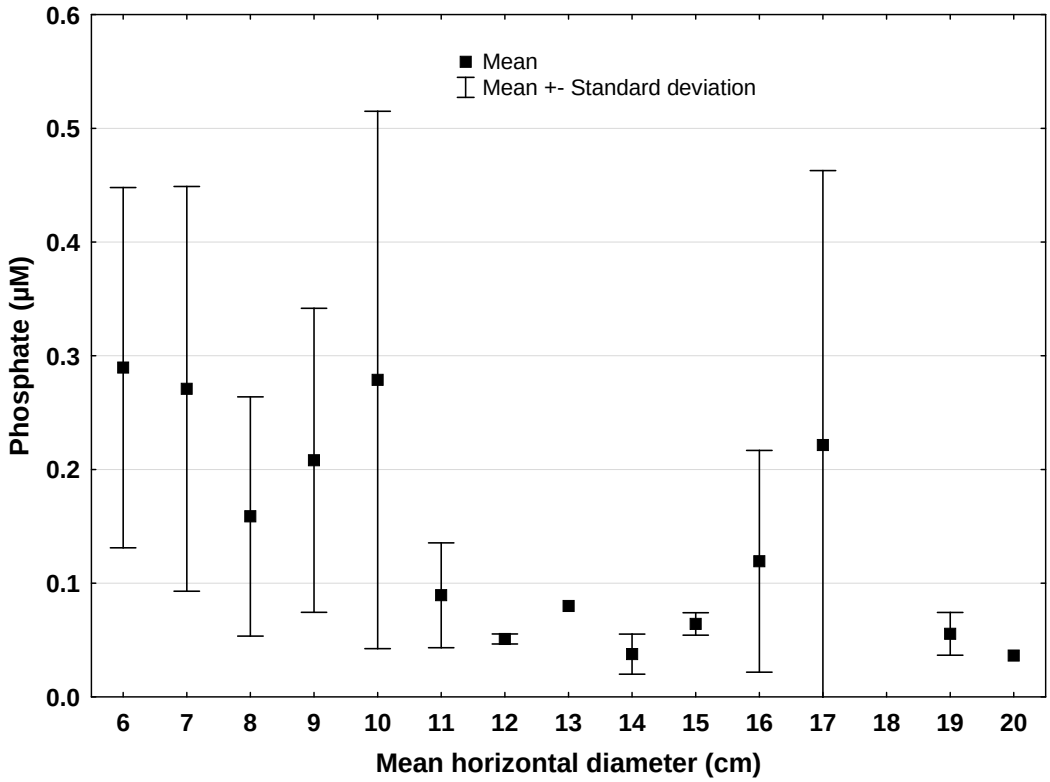


Figure 22 – Phosphate concentrations in the lumen of *Codium elisabethae* versus algae class size, data aggregated over season and sites.

6.3.5 Nutrient concentration ratio between lumen water and sea water

The nutrient content measured in the lumen water of the macroalga (as described above) can be compared with nutrient concentrations measured in the sea water surrounding these populations (see Chapt. 5).

The global nutrient concentration factor in the lumen of the *Codium elisabethae* populations studied are demonstrated to be important (NO3: 5.7; NH4: 3.4; PO4: 3.1) (Table 2), as it was demonstrated for *Codium bursa (*Geertz-Hansen *et al.*, 1994; Vaqué *et al.*, 1994; Vidondo *et al.*, 1995 and 1998) with nutrient ratios ranging from 1 to10 according to seasons. On samples of *Codium elisabethae* from Monte da Guia, as of *Codium bursa* from Calvi (Corsica), various species of *Cyanophyceae* were found entangled at the lower internal face of the thallus. The role of these species in a symbiotic relation to *C. elisabethae* and *C. bursa* by nitrogen fixation can be suggested as the isolated laboratory culture of these internal cyanobacteria (blue-green algae) turned difficult in nutrient depleted media (Demoulin, personal communication). This would be consistent with observations by Rosenberg and Paerl (1981), demonstrating lower nitrogen concentration in *Codium decorticatum* within which the nitrogenase activity of cyanobacteria (*Calothrix* sp., *Anabaena* sp., and *Phormidium* sp.) was inhibited.

These concentration ratios are higher for the population of Ponta Furada than of Caldeirinhas for nitrate, ammonium and phosphate (Figs. 23, 24 and 25). Considering the trend to lower global seawater concentrations in the site of Ponta Furada, these higher global concentration ratios as absolute lumen nutrients concentrations measured in the population of Ponta Furada is an argument to understand the particular shape of this macroalga as an efficient functional adaptation to oligotrophy.

Table 2 - Mean nutrient concentrations, and concentration ratio between lumen of *Codium elisabethae* and seawater

µM	NO_3^-	NH_4^+	PO_4^{3-}
lumen	4.73	1.92	0.22
sea water	0.84	0.57	0.07
concentration ratio	5.65	3.38	3.10

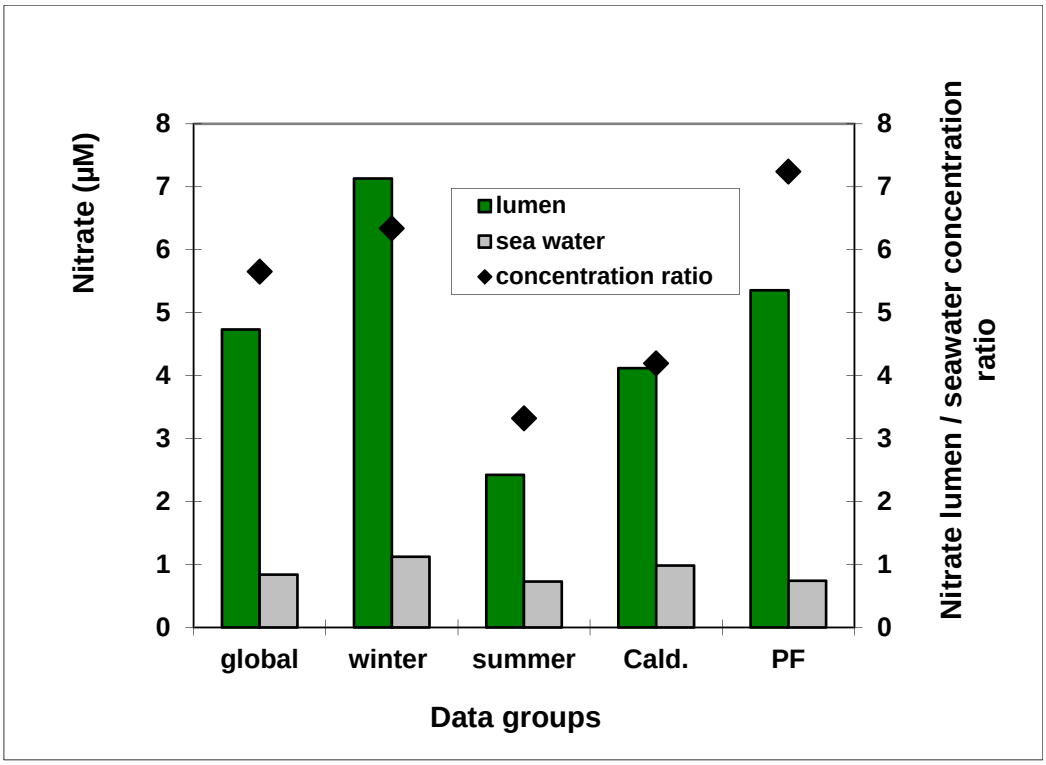


Figure 23 – Global nitrate concentrations in sea water and in *Codium elisabethae* lumen water, with ratio.

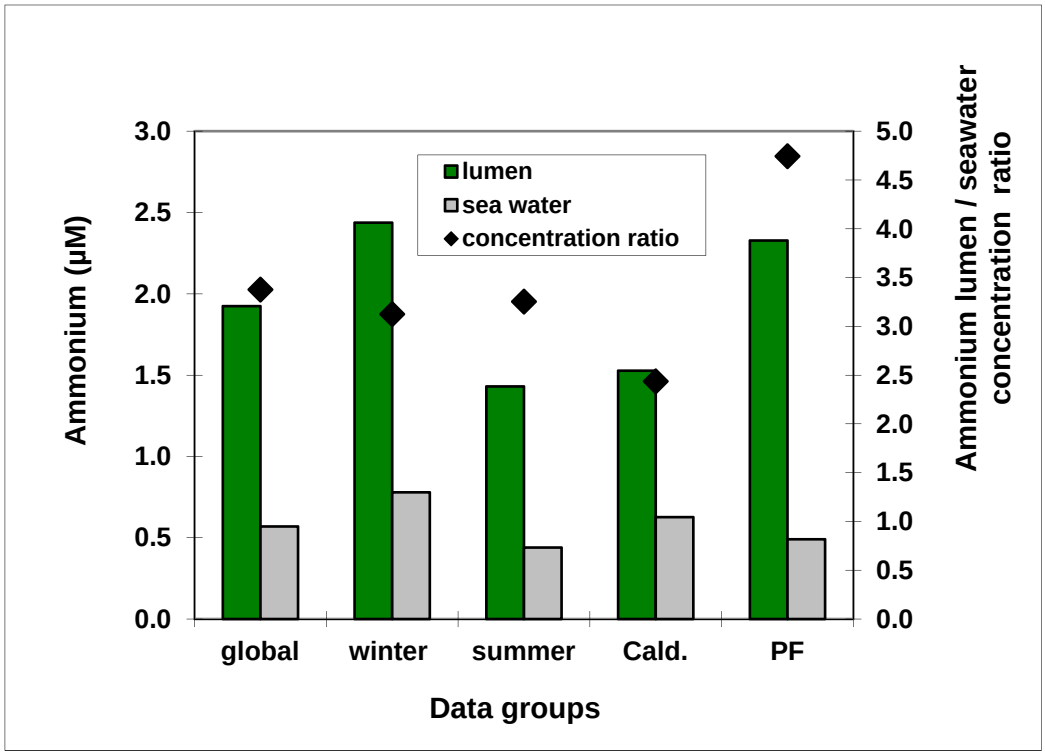


Figure 24 – Global ammonium concentrations in sea water and in *Codium elisabethae* lumen water, with ratio.

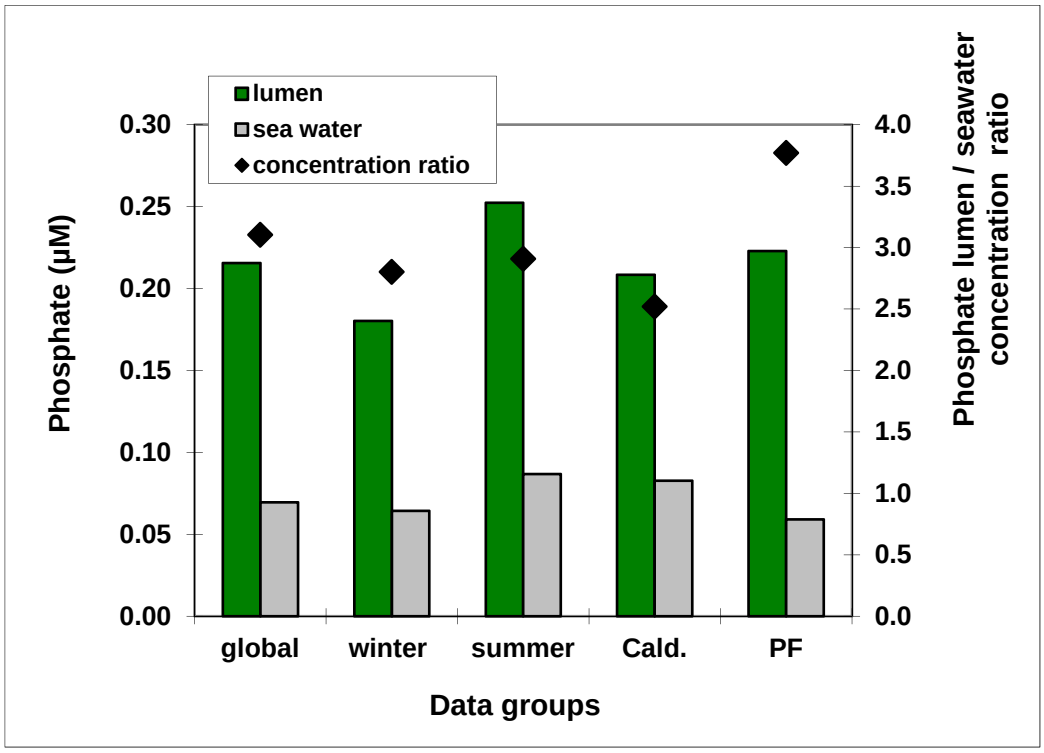


Figure 25 – Global phosphate concentrations in sea water and in *Codium elisabethae* lumen water, with ratio.

6.3.6 Epiphytism on *Codium elisabethae*

Epiphyte biomass measured on *Codium elisabethae* increases globally with their age and diameter and seems to present a seasonal dynamics as revealed by measures carried out in winter and summer 2004 on both Ponta Furada and Caldeirinhas populations (Fig. 26). For both sites, epiphyte biomass reach highest values in summer than in winter, and this becomes significant when evaluated for individuals larger than 12 cm. The maximal values of epiphytism are found in Ponta Furada for both seasons.

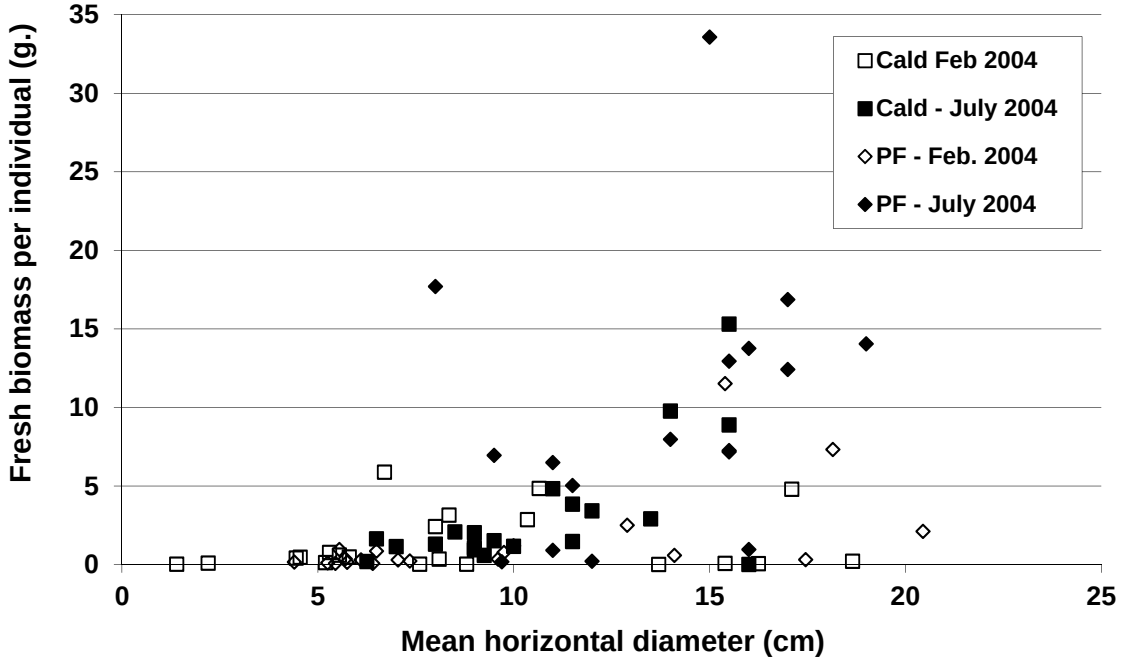


Figure 26 – Total epiphyte biomass on *Codium elisabethae* versus mean horizontal diameter, measured during winter (Feb 2004) and summer (July 2004) in both sites.

6.4 Conclusions

Density, size structure, recruitment, patchiness

The *in situ* countings confirmed the general differences of population structures through time: higher density and more continuous size distribution for Caldeirinhas population, whereas bigger individuals are more frequent in the site of Ponta Furada, and reach larger maximal size than in the Caldeirinhas. An important spatial variability of structures and densities at small spatial scale was demonstrated within each of the sites, both during summer and winter periods. Mean density of young recruits produced by sexual reproduction was clearly higher in the site of the Caldeirinhas than in Ponta Furada. This higher density of young recruits in the Caldeirinhas can be explained both by the higher reproduction investment observed for that population and by a probable higher settling success of germling stages. The low density of recruitment remained almost constant in Ponta Furada, whereas more intense spatio-temporal dynamics was observed in Caldeirinhas.

Morphology

The flattening shape evolution is confirmed as for *Codium bursa* (Geertz-Hansen *et al.*, 1994), and shown to be more pronounced for the Ponta Furada population. This is probably a consequence of lower intra-specific competition for space or a natural selection due to higher average current speed in this site.

Sexual reproduction

Based on populations sampled in reference sites within the SAC of Monte da Guia, Azores, this research produced the first extensive quantification of the dynamic of reproduction for the *Codium elisabethae* species. The density of utricles with gametangia was shown to be significantly higher in December than in February and summer periods. In summer, the reproduction is less intense than in winter, but it is not absent, in contrast with earlier observations made for the *Codium elisabethae* populations of the Azorean Island of Sao-Miguel (Neto, 1997). It is also different than mentioned by studies on other *Codium* species: gamentangia were found from for *Codium bursa* (Geertz-Hansen *et al.*, 1994; Vidondo and Duarte, 1995) and from June to September for *Codium fragile* (Bulleri *et al.*, 2007). This information is an important confirmation of the active summer reproduction suggested by our *in situ* observations of numerous very young recruits during summer. The density of utricles carrying gametangia tends to be higher for the population of the Caldeirinhas for each period, but no significant statistical differences could be demonstrated with the available data. When aggregating these data for both sites, a general positive correlation between density of utricles with gametangia and mean horizontal diameter is shown to be statistically significant for all periods tested. Generally, we observe an earlier onset of sexual reproduction comparatively to the one of Ponta Furada

Vegetative reproduction

For both sites, the intensity of vegetative reproduction appeared not significantly affected by the season of observation, whereas for each collecting period, the vegetative reproduction is significantly more intense for the population of the Caldeirinhas. For each site, the diameter of parent alga was shown to have a significant effect on the total number of propagules carried. This mode of reproduction can be described in two phases according to the algae life. A first propagule production phase occurs during an early life stage, followed by a growth period with almost absent propagule production (from 9.5 till 11.5 cm in the Caldeirinhas and from 11 till 15 cm in Ponta Furada). Then a second vegetative reproduction phase is observed

Nutrient concentrations in the internal water (lumen)

In summer, nutrients concentrations are non-significantly different between the populations of both sites. In winter, all internal water nutrients concentrations tend to be superior for the population of Ponta Furada, but the difference is only significant for ammonium. In the Ponta Furada population, summer concentrations are all significantly lower than in winter, whereas in the Caldeirinhas population the summer concentrations are only slightly reduced as compared to winter situation, and the difference is only barely significant for the nitrate.

***Codium elisabethae* lumen to seawater nutrient concentration ratios**

This work provides a first evaluation of the nutrient concentration ratios existing between the lumen water of *Codium elisabethae* populations and their surrounding sea water. These global nutrient concentration ratios are demonstrated to be important (NO3: 5.7; NH4: 3.4; PO4: 3.1), and is comparable to the ones demonstrated for *Codium bursa* in the Mediterranean Sea (Geertz-Hansen *et al.*, 1994; Vaqué *et al.*, 1994; Vidondo *et al.*, 1995 and 1998). These concentration ratios are higher for the populations of Ponta Furada than for the ones of Caldeirinhas. Considering the global trend to lower concentrations in Ponta Furada seawater, the higher absolute lumen nutrients concentrations and global concentration ratios measured in the population of Ponta Furada constitute an argument to understand the particular shape of these macroalgae as an efficient functional adaptation to oligotrophic conditions.

Epiphytism on *Codium elisabethae*

For both sites, the epiphyte biomass is higher in summer than in winter for individual larger than 12 cm. The maximal values of epiphytism are found in Ponta Furada for both seasons.

6.5 References

Bulleri, F., Branca, M.G., Abbiati, M., and Airoldi, L., 2007. Development of reproductive structures in the introduced green alga, *Codium fragile* ssp. *tomentosoides*, in the northern Adriatic Sea. European Journal of Phycology, 42(2): 137-144.
doi: 10.1080/09670260701231243

Desclin, P., 2005. Etude comparative des macroalgues *Codium elisabethae* (Açores) et *Codium bursa* (Corse): capacités de concentration des nutriments et cycles de reproduction. Mémoire de Maîtrise en Océanologie, Université de Liège, 51 pp.

Geertz-Hansen, O., Enriquez, S., Duarte, C.M., Agusti, S., Vaque, D. and Vidondo, B., 1994. Functional implications of the form of *Codium bursa*, a balloon-like Mediterranean macroalga. Marine Ecology Progress Series, 108: 153-160.

Neto, A.I., 1997. Studies on algal communities of São Miguel, Azores, PhD thesis in Marine Biology, University de the Azores, Ponta Delgada.

Neto, A.I., 2000. Observations on the biology and ecology of selected macroalgae from the littoral of São Miguel (Azores). Botanica Marina, 43: 483–498.

Rosenberg, G. and Paerl, H.W., 1981. Nitrogen fixation by blue-green algae associated with the siphonous green seaweed *Codium decorticatum*: effect of ammonium uptake. Marine Biology, 61: 151-158.

Vaqué, D., Agusti, S., Duarte, C.M., Enriquez, S., and Geertz-Hansen, O., 1994. Microbial heterotrophs within *Codium bursa*: a naturally isolated microbial food web. Marine Ecology Progress Series, 109: 275-282.

Vidondo, B. and C.M. Duarte, 1995. Seasonal growth of *Codium bursa*, a slow growing Mediterranean macroalga: *in situ* experimental evidence of nutrient limitation. Marine Ecology Progress Series, 123: 185-191.

Vidondo, B. and C.M. Duarte, 1998. Population structure, dynamics and production of the Mediterranean macroalga *Codium bursa* (*Chlorophyceae*). Journal of Phycology, 34(6): 918-924.
doi: 10.1046/j.1529-8817.1998.340918.x

7 - *Codium elisabethae* population structure and dynamic inferred by seabed visible imagery

Table of contents

7.1 Introduction and objectives

The main objective of this research is to quantify precisely the dynamic of *Codium elisabethae* macroalga populations within the Special Area of Conservation (Natura 2000 network) of Monte da Guia (Faial), as listed in the 2005 report of the Working Group on Habitat Mapping (WGHM), International Council for the Exploration of the Sea (ICES, 2005).

This approach was initiated by first exploratory studies using manual recognition based on video transect (Salgado, 2002; Salgado *et al.*, 2001a, 2001b, 2002), then using automated methods (Sirjacobs, 2002), and followed by refined methodology development (Sirjacobs *et al.*, 2004a, 2004b and 2006) resulting in the long term monitoring of reference sites presented here. In order to improve the quality of seabed mosaics and of the population estimations produced for the long term survey over our referenced study sites, various improvements were brought progressively to the image acquisition process. This technical search for the most efficient global method required many interactive changes at various levels, each of them imposing direct adaptations in the whole chain linking material, method and data processing. Thus, the whole methodology finally set up (the material selected, the method of shooting images, the software tested or developed for processing) is in itself a result.

The analysis exploits a two year time series (August 2003 - November 2005) of metrically corrected photo-mosaics collected in scuba diving in two sites along Faial south coast to extract parameters such as seasonal fluctuations of density, percentage cover, biomass, growth rate and primary production of the species. In addition to represent the first quantitative evaluation of the *Codium elisabethae* growth rates and primary production, this research tests the potential of visible imagery mosaic mapping for detailed long term, frequent and spatially continuous quantification of benthic biological processes at high resolution.

This study produced a spatially referenced and geometrically corrected image mosaics database exploitable as a basis for any future researches on the spatial organisation dynamics of *Codium elisabethae* and of other species occupying the studied Azorean benthic habitats.

7.2 Methods

7.2.1 Data collection

7.2.1.1 Sites

In the marine Natura 2000 SAC of Monte da Guia (Faial Island, Azores, NE Atlantic), two study sites were selected (Fig. 5, Chapt. 2) to investigate the specific dynamics resulting in contrasting *Codium elisabethae* population structures and densities. One site is located in the sheltered no-go reserve of the Caldeirinhas, constituted by ancient volcano craters, now eroded and half opened to the sea. By 23 meters depth, the extensive rocky bottom of the outer crater is occupied by a dense *C. elisabethae* population. The second site is in a location experiencing more exposed conditions, at a distance of about 2 km from the first one. There, a more sparse population occupies rocky tables and boulders emerging at about 19 meters depth from shallow sandy deposits (site of Ponta Furada).

Study sites were identified as rectangles of 8m length by 4 m width. White acrylic squares were chosen as permanent seabed marks to allow for image geo-correction during photo mosaicking. After preliminary in situ tests devoted to define the range of possible distance to seabed for image acquisition, the size of the reference marks were set as squares of 10 by 10 cm, so as they would remain well distinct from background even on the most remote images. These marks were fixed into the volcanic rocky substrate with non-oxydable nails (Figure 2). Much efforts were required to maintain the network of marks in place and useful. Regular cleaning of the biotic colonization was necessary to keep the marks well visible and many of them had to be replaced after currents and wave effects had broken or taken them away.

Considering the required resolution, the camera angle of view, the generally weak luminosity and contrasts obtained at these depths and the seasonal turbid events, corners and intermediate marks were disposed on vertices of a 2 m quadrangular grid (Figure 22). After the fixation of marks, real distances and depths differences between each mark were measured in scuba diving with a soft decameter line graduated at centimeter intervals, to allow the estimation of relative horizontal coordinates of the center of each mark (see section 7.2.2.1). This work was and performed again when some broken or advected reference marks had to be replaced.

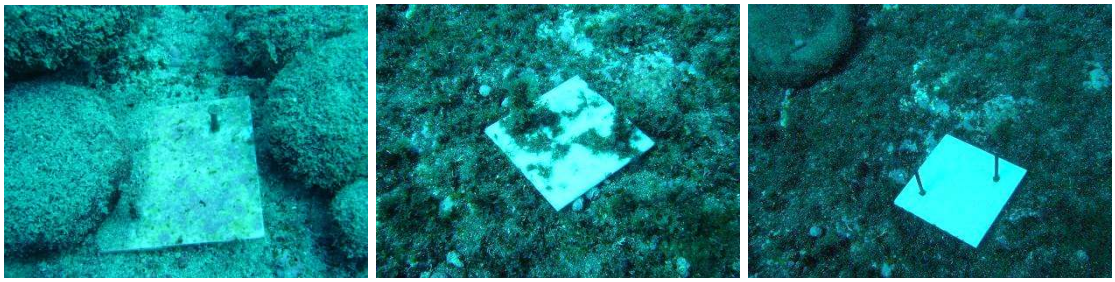


Figure 1 - Acrylic plate nailed in the rock as reference mark for geometric correction of seabed images (left and center: colonized; right: cleaned plate).

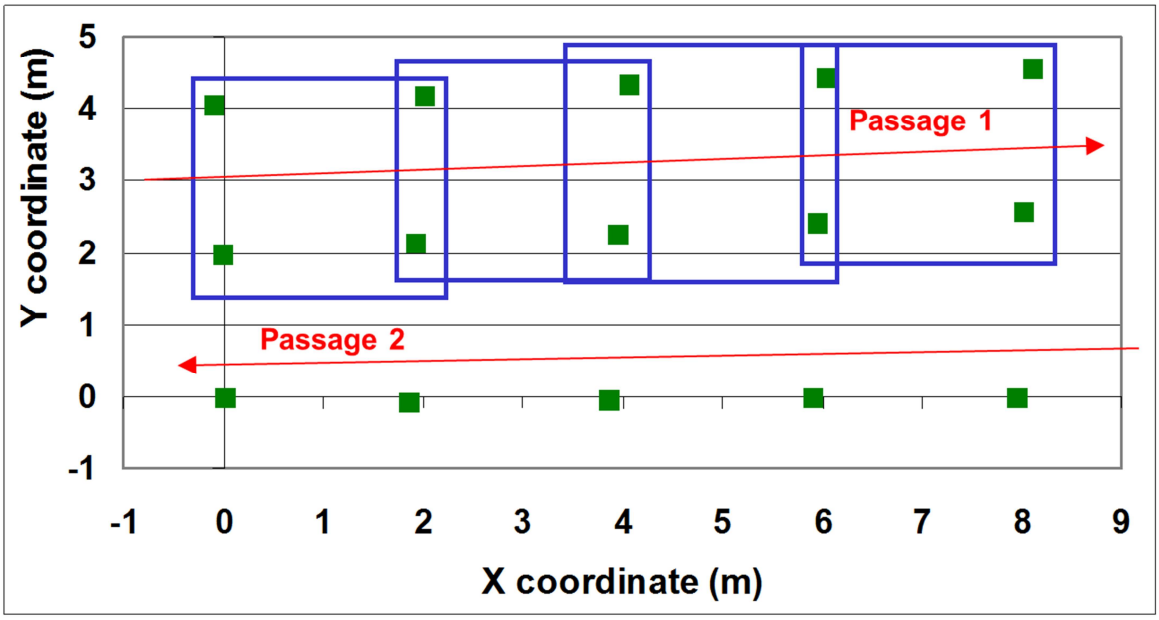


Figure 2 - General scheme of the installation of reference marks delineating study site; example of the Caldeirinhas; green square = reference mark, red arrow = scuba diving passage 5 m above seabed; blue quadrat = individual image footage including 4 reference marks.

7.2.1.2 Data acquisition methods

The aim of the imagery methodology developed is to increase the practically accessible spatial scale and temporal frequency of population monitoring in benthic environment, as well as to conserve visual record of position and shape of benthic organisms or objects, in order to monitor temporal variations based on precise spatial geocoding of seabed image mosaics. Imagery was collected continuously in scuba-diving by covering the study sites at controlled distance to seabed. During a preliminary phase of the study, both digital video and still cameras were used. Video imagery collection was conducted using a semi-professional digital camera Sony DCR-VX1000E (featuring a 3 CCD sensor with a 0.4 megapixel resolution) mounted on a diving scooter. The continuation of field surveys allowed to compare the quality of mosaics produced from video footage with photomosaic produced from stills taken with a commercial Sony DSC-P9 digital camera (single CCD sensor with resolution of 4 megapixels). Compromised had to be searched between reducing distance to seabed for increasing image quality (high pixel resolution and low light diffusion), and increasing distance to seabed for increasing extent of seabed coverage and reduce mosaicking difficulties. After preliminary surveys, the optimal quality of mosaics was obtained using still images taken at a 4 m distance. No artificial lighting was used. Imagery surveys were carried out preferentially between 11h00 and 15h00 with clear sky and calm sea. Zooms of camera were set for maximum wide angle.

Along the study, 42 repetitions of *in situ* counting and measurement were produced to provide a basis of validation for the results obtained with imagery, and to produce a closer estimation of younger individuals for a recruitment study. The methodology consisted of placing a 1 by 1 meter thin metallic quadrat on the bedrock, its corners being positioned over nails fixed in the rock, within the study sites devoted to visible imagery experiments. A close photo of the quadrat in place was taken for good correspondence localization within the larger image mosaics produced. The measurement of the diameter of all individuals presents was done by a first diver with a caliper (mm precision) and with a 0.5 cm graduated meter band for

individuals larger than 22 cm. Subdivision of the zone was made easier by the use of a gridded smaller metallic quadrat, and measures were communicated to a second diver for hand recording. For ovoid shaped thallus, both long and small horizontal diameters were recorded for estimation of a "mean horizontal diameter" as seen from above by imagery. Limited by to scuba diving imperatives (depths of 20 to 23 meters), the time consuming counting was possible for a maximum of three square meters per dive in Ponta Furada, and to only one square meter for the more densely populated site of the Caldeirinhas. Based on good position identification of the quadrats within the general mosaic and on the short time lags between the *in situ* counting and the closest image acquisition, 20 *in situ* counting were selected to evaluate the consistency of the population structure produced by either *in situ* or imagery-based estimation methods.

For both study sites, sampling and subsequent laboratory measures were performed all along the study based on a total of 100 individuals collected in the vicinity of both study sites during the field campaigns of the summer 2003, winter 2004 and summer 2004. The objective is to establish experimental relation between the mean horizontal diameter and the biomass of individuals, so as to provide the possibility of an indirect estimation of the spatially integrated values of biomass from the population size structure obtained by image analysis. Standard procedures were followed to evaluate fresh biomass of individuals after draining all lumen internal water by gravity, and later transformation into dry biomass was based on the weight ratio obtained after drying 12h in 50°C oven.

7.2.1.3 Dates of imagery surveys

Regular imagery monitoring surveys started in the end of August 2003 and lasted until November 2005. Image mosaicking and subsequent processing could be established for 15 periods for the site of Caldeirinhas, and 17 periods for the site of Ponta Furada (table 1). These data were processed to produce a description of population dynamics over more than 2 years.

	Ponta Furada		Caldeirinhas
1	21/8/03	1	17/8/03
2	10/10/03	2	9/10/03
3	21/11/03	3	21/11/03
4	26/2/04	4	27/2/04
5	27/7/04	5	27/7/04
6	18/8/04	6	11/8/04
7	4/10/04	7	18/8/04
8	28/10/04	8	20/10/04
9	26/11/04	9	23/11/04
10	9/12/04	10	3/3/05
11	9/2/05	11	11/7/05
12	3/3/05	12	26/7/05
13	14/7/05	13	19/8/05
14	26/7/05	14	28/8/05
15	19/8/05	15	11/11/05
16	27/8/05		
17	11/11/05		

Table 1 - Date list of exploited imagery surveys

7.2.2 Data processing

Subsequent processing consisted of (1) estimating relative positions of reference marks, (2) producing image mosaics, (3) using interactive detection of individuals present, (4) showing valid correspondence of *in situ* with imagery based population size structure detection, running automated processing yielding (5) population dynamical parameters from image change detection, and (6) inferring biomass and primary production estimates over the whole imagery sites and the 2 years monitoring period.

7.2.2.1 Topography of reference marks

Relative horizontal coordinates of the center of reference marks were estimated by combining all *in situ* distance measurements in a graphic representation with a computer assisted drawing software (AutoCad; © Copyright 2011 Autodesk, Inc.) (Figure 23).

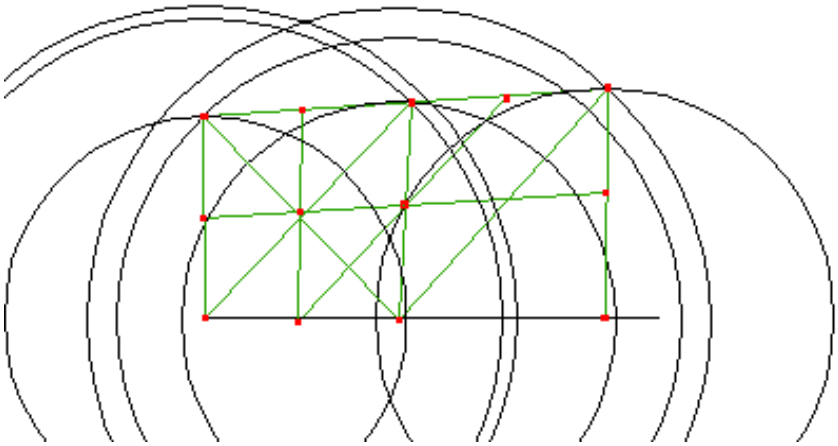


Figure 3- Illustration of relative reference marks positions reconstruction (red) by progressive compilation of in situ horizontal distance measures (green lines and black circles) into a computer assisted drawing software (site of Caldeirinhas).

The coordinates of the center of reference marks were taken as the intersection of redundant distance measurements. When necessary, the center of the polygon defined by distinct intersections of redundant distance measurements was taken as the center of the reference mark (Figure 24), thereby geometrically minimizing the maximal error on relative position estimation between two reference marks, by supposing an equivalent contribution of every distance measurement to the final positioning error. The maximal error on relative position between two plates is estimated as the maximal distance between mark center (x_c,y_c in figure 4) and any surrounding distance intersection (x_1,y_1; x_2,y_2; x_3,y_3 in figure 4) was observed to fall below 2 cm, which is below 1 per cent of mark to mark nominal distance.

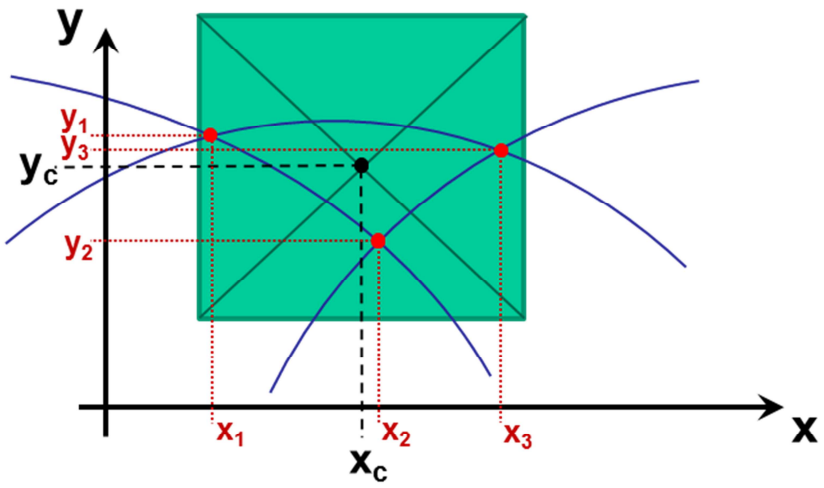


Figure 4- Coordinate selection scheme for the center of a reference marks (green square); taken as the center of the polygon defined by intersections (red dots) of redundant distance measurements (blue arcs).

The result of this graphical approach was considered precise enough for our application and no global analytical processing was set up to produce an optimized repartition of the measurement errors. Such topographical algorithms do exist and are nevertheless suggested, together with the use of measurement with rigid structure propagation, for future improvements of the global methodology. This will optimize final precision and mainly allow automation of heavy processing of error repartition, particularly useful if the metric of the targeted mosaics requires higher precision for the study of smaller organisms.

The experience of mosaic reconstruction (quality of the continuity at image transition zones) based on these reference coordinates had shown that for the site of Ponta Furada, the real horizontal distance between reference marks to consider in the reconstruction should be computed prior from non-horizontal distances measured underwater and relative depth variation in order to produce marks coordinates with acceptable precision with the graphic method used. This is due to a more pronounced topography irregularity of this site, as shown in Annex 10.7.

7.2.2.2 Mosaicking

The mosaicking process consisted of merging several images into a single one, creating thus a panoramic view (Gracias and Santos-Victor, 2000; Garcia-Campos, 2001; Singh *et al.*, 1998). Here, our specific goal was to define a mosaicking methodology that permitted rectified images to be used for extracting *Codium* metrics. For both video and still image sequences, various methodologies were investigated that included commercial stitching packages and specifically developed Matlab codes.
Video mosaicking was conducted with the early versions of Adelie Mosaic package (©Ifremer; Ifremer, 2010) and with the Mosaico.m Matlab software (Costeira, personal communication). Both softwares allow adjusting parameters to optimize image fusion and improve the final result. For the present application, Mosaico.m provided better results than Adelie due to enhanced control access to source code parameters. Due to variations in light conditions and in the roll and pitch of the camera, some distortions and discontinuities can appear in the final mosaics (Fig. 5a and Annex 10.4). The high image acquisition rate of video

footage provides more images than required as far as the minimum overlap needed to stitch successive images is concerned. However, with the exploited mosaicking software's, this reduced the average quality of the mosaics due to the higher sensitivity of the resulting imagery to camera movements. Whilst a few blurred images do not disturb human eye at regular frame refreshment rate, the same type of faults strongly impacts on the mosaicking processes used in both software's, generating a progressive image deformation that prevents precise spatial rectification. Both codes allow sub sampling the video sequence in order to minimize the computationally heavy calculations conducted to stitch adjacent images, but automated blurred image detection and discarding from the geo-corrective projections process would require important changes in the software coding and heavier computing for uncertain results.

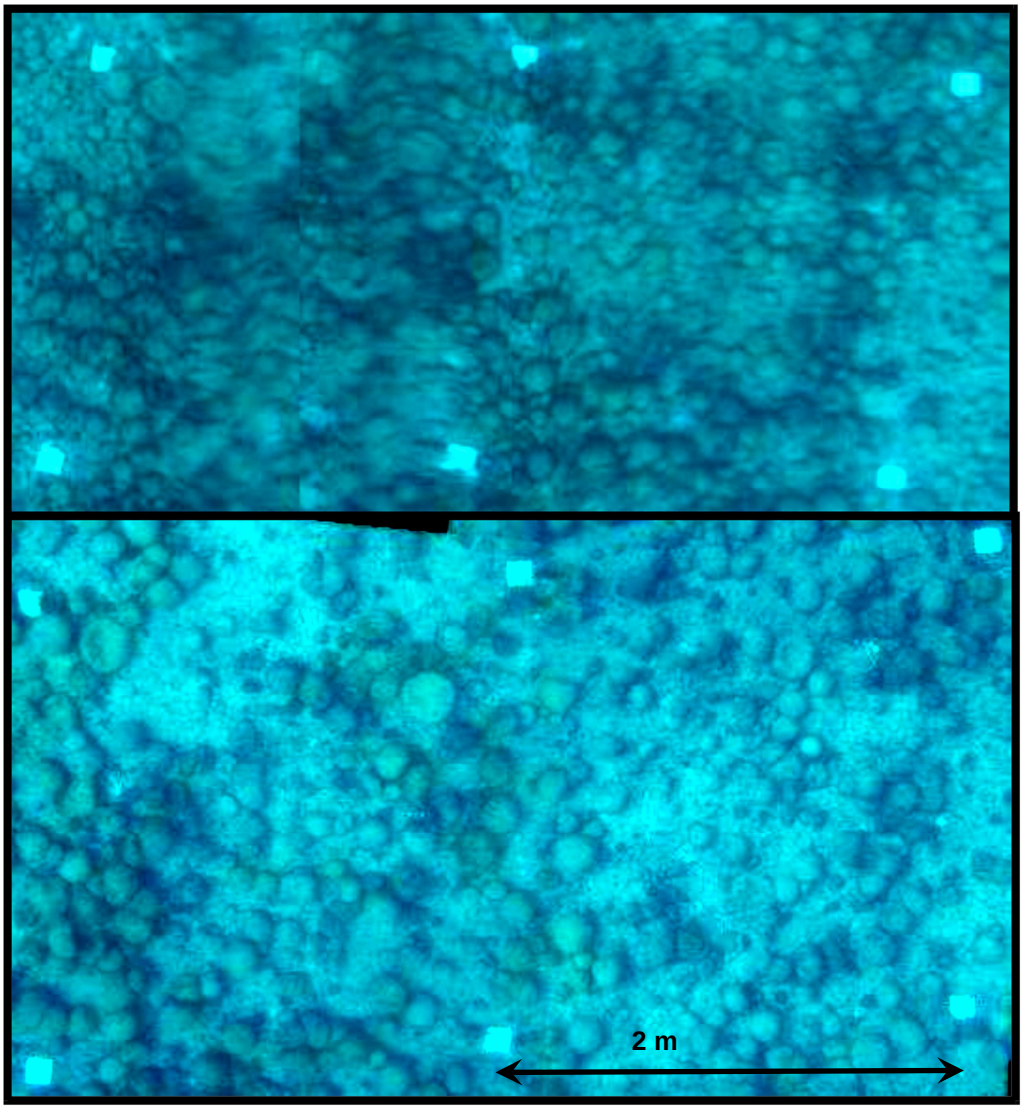


Figure 5 - a) Zoom into a mosaic build from video imagery showing dense fields of macroalga *Codium elisabethae*, classical blur deformation zones appearing. b) Zoom into a mosaic build from still camera image over the same zone and timing as in (a); both areas cover 2 by 4 meters.

The advantages of still imagery were (i) an enhanced resolution of the seabed, (ii) a reduced number of images to select and process, (iii) a better focus due to direct control of speed and depth during image acquisition. Two commercial software packages were tested to build the mosaics: Canon Photostitch (© Canon) and the ArcView GIS georeferencing tool (© Esri).

Photostitch was fast and generally produced very good fusion at the image overlaps. However, since it did not permit introducing any georeferencing correction, mosaics showed large distortions from the actual geometry and relative dimensions of the sites (Annex 10.5). ArcView had the advantage of providing graphical and numerical control tools for georeferencing each image individually, but did not allow any control over image cropping and fusion and is heavy in terms of applications involving repetitive encoding of similar coordinates, so not adapted for the numerous operations in long term monitoring applications.

A Matlab program was thus written to produce referenced and geometrically correct mosaics. In order to build each mosaic, the program requires the user interaction to select, in zoomed sub-views, the pixels corresponding to the center of each of the four reference marks present in each input image, in a clockwise order starting from the top left mark. Then, the algorithm exploits automatically the coordinates of the four concerned reference marks (section 7.2.2.1) to apply an independent affine transformation which projects each pixel to the expected final x,y coordinates and resamples the final image on a regular grid of 2 mm, the mean spatial resolution of the input image. The general mosaic is progressively constituted by the compilation of successive geo-corrected images in a general matrix, adding new information only where no pre-existing data was brought by the processing of earlier images. The good image continuity obtained by this non-blending process at image transition zones confirms the satisfying positioning process. Better mosaics were thus obtained from our still images with this specifically developed code, and in a far more efficient way (Fig. 5b and Annex 10.6): without blur deformations, without stitching discontinuities, with higher resolution and metrically corrected projection. Relative differences of elevation had to be taken into account for horizontal distance correction in the site of Ponta Furada which shows a more irregular topography (Annex 10.7), in order to minimize consequences of perspective deformations and allow exploitable final mosaic as illustrated (Annex 10.8 and 10.9).

7.2.2.3 Detection of individuals and population structure

Early work was devoted to the development of an algorithm allowing the automated detection of *C. elisabethae* individuals from video mosaic transects conducted on the Caldeirinhas site (Sirjacobs, 2002). Once all the parameters were tuned and provided visibility and light conditions were good, the algorithm showed high detection efficiency. However, seasonal variations of lightning conditions, water turbidity, epiphyte growth and sediment layer turned the optimization of detection parameters so laborious that human-assisted detection represented a more efficient approach. The detection program was thus adapted so as to display a false color zoomed image (based on blue and green bands equalization) of successive subzone (1 m²) to process and request the interaction of the user to position the center and edge of each identifiable *C. elisabethae* by pixel selection. Successive sub-zones of 1 m² are processed this way till covering the whole mosaic, while location and size records are compiled by the code. The program then provided automatically the number of individuals, the size histogram and the percentage of substrate covered by the *C. elisabethae* population. The detection results are finally merged into a general view of the distribution of individuals at the scale of the complete mosaic studied. Figure 6 illustrates such results for a 4 by 4 meters portion of the seabed in the sites of Caldeirinhas and Ponta Furada.

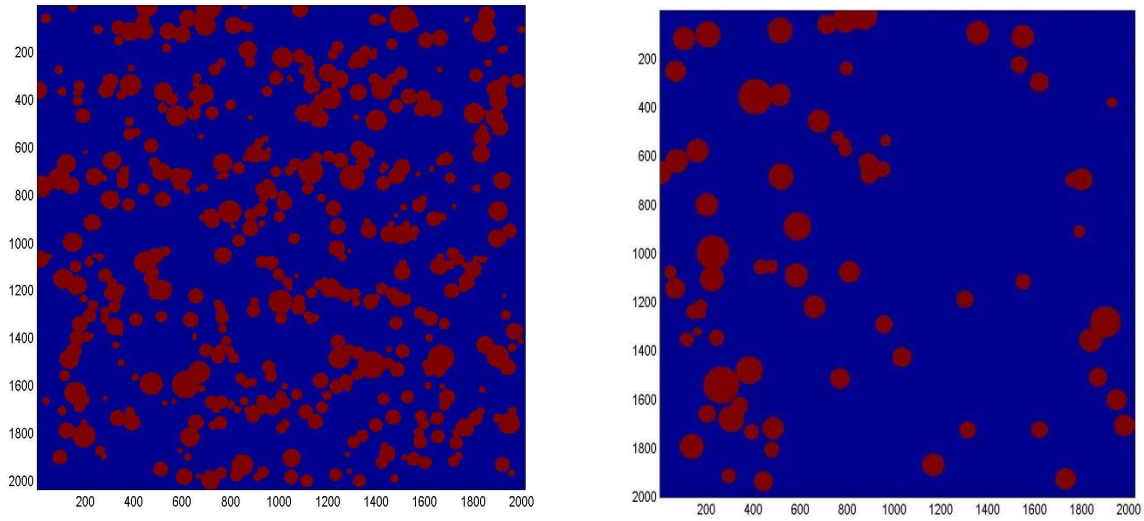


Figure 6 - Illustration of *Codium elisabethae* detection result (red disks) for half of the site of Caldeirinhas (left) and Ponta Furada (right), each picture (graduated in pixels) represent 4x4 meters (pixel resolution=0.2 cm).

7.2.3 Validation of population structure with *in situ* measurements

The Chi-square contingency table analysis was used to compare the size histograms obtained by either *in situ* or imagery-based estimation methods. The size histograms of validation counting were built with a 1 cm class size for each detection method. The test was applied as presented in Zar (1999), respecting the guidelines given by Roscoe and Byars (1971) to avoid introducing any bias in the analysis. These guidelines mention that the analysis is free of bias if the average expected frequency (total number of data divided by the product rows*columns) is superior or equal to 6.

7.2.3.1 Population dynamics parameters from image change detection

Similar mosaicking and detection analyses were executed for the successive monitoring surveys. In the site of Ponta Furada, population change detection was achieved over the whole site (32 m²), while in the Caldeirinhas, a reduced area of 16 m² was exploited due to the very high population densities. They showed the evolution of the population not only in terms of size structure (as an *in situ* counting would provide) but moreover of the establishment, growth and disappearance of specific individuals (Fig. 7). Two Matlab codes were designed to process automatically all the successive detection results as follows. A first processing synthesized the temporal evolution of direct and indirect observations such as number of individuals, density, substrate percentage cover and mean diameter. It also created images of "differences between two periods", providing color codes that allowed easy visualization of changes. A second processing phase exploited images produced by the first step (uni-temporal and change detection images) to test the change in each individual and classify it as new, vanished, or growing. This process could thus provide an automated quantification of growth rate of individuals according to size, period and site studied. Similarly, mortality or "recruitment" of individuals may be produced.

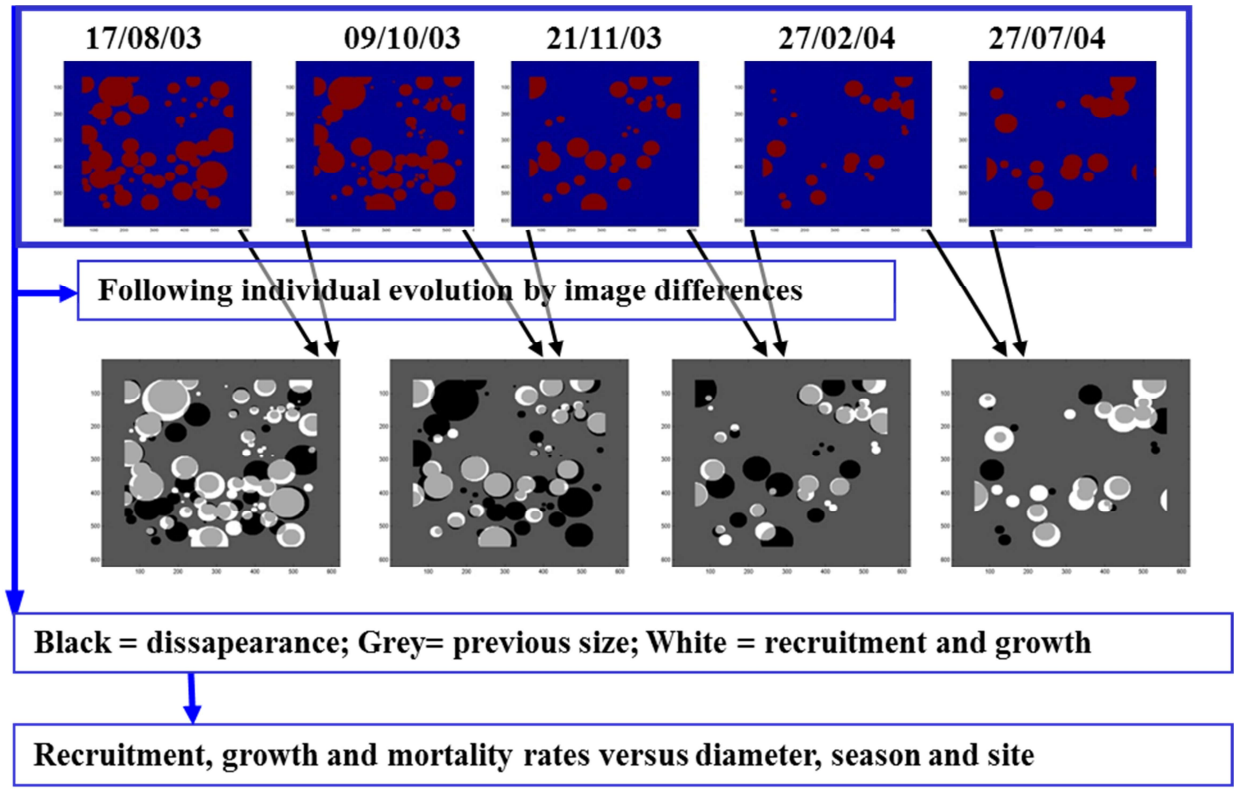


Figure 7 - Illustration of the possibility to follow by image differences the evolution of individuals detected (red disks) in successive mosaics (1 m² extracted from Caldeirinhas time series).

7.2.3.2 Estimation of biomass and primary production from imagery

Based on the relation established in laboratory between the biomass and mean horizontal diameter of *C. elisabethae* individuals (Fig. 7 in Chapter 6), the algae sizes measured by imagery can be converted by the imagery codes into biomass estimates, producing mean biomass values (g. fresh weight/m² or dry weight/m²) for each site and imaging period.
The imagery method provides more representative biomass estimation than *in situ* measurements as it can integrate data from a larger area (considering work time invested), allowing to average out the artifacts of some smaller-scale patchiness. Imagery also allowed to derive better insight into questions of primary production than would have been possible by *in situ* measurements, which would convey at best the evolution of the global population size histogram. Estimating primary production just from temporal changes of population size histograms would leave some uncertainty as, *a priori*, several combinations of recruitment, growth and mortality rates can lead from one population histogram to the next. This uncertainty is eliminated with seabed imagery methods allowing to follow up the evolution of each individual from initial to final diameter, as long as temporal frequency of imagery surveys is adapted to resolve the growth dynamics. Thus, primary production were calculated for each site and each period separating two imagery surveys, with a distinction of the contributions to primary production linked with (1) the establishment of young individuals, (2) the primary production resulting from the growth of existing individuals, and (3) the exportation of biomass due to mortality/removal (grazing or advection); (Figs. 17 and 18).

7.3 Results and discussions

7.3.1 *Codium elisabethae* biomass - size relation

The evolution of the thallus biomass was modelled by a power relation of an order superior to square, reflecting the fact that the thallus thickness increases also during the alga growth, while the alga morphology changes from spherical-like in younger stages to variably flattened discoid at later stages (Fig. 7 in Chapter 6). The average ratio measured in laboratory between dry (g. dry weight) to fresh biomass (g. fresh weight) of *Codium elisabethae* thalli is of 0.068, and was used for conversions in later processing's.

7.3.2 Validation of the imagery approach developed

Contingency analysis (tables in Annex 10.10) shown that for the Caldeirinhas alone, the 1 cm precision class size histogram produced by imagery method can be considered equivalent to the *in situ* counting (non-significantly different), if taking into account the classes of size above 4 cm (average expected frequency AEF=7.54 > 6; Chi^2= 12.43; dl=20; $p<0.99$). In other words our imagery approach applied to the topographic conditions encountered in the Calderinhas is providing a valid, centimeter precision estimation of population structure, for individuals which are above 4 cm diameter. For Ponta Furada alone, the whole histogram cannot be tested in one piece as the associated average expected frequency is too low (AEF<6) due to the lower effectives per class-size resulting from lower population density, although a three-fold larger surface was apprehended for validation in this site. Nevertheless, it was shown that 1 cm precision class size histogram produced by imagery method can be considered equivalent to the *in situ* counting (non-significantly different), if taking into account classes of size between 5 and 16 cm, for which the previous test validity criteria is acceptable (average expected frequency AEF=6.136 > 6; Chi^2= 5.263; dl=10; $p<0.873$). In other words our imagery approach applied to the topographic conditions encountered in the Ponta Furada is providing a valid, centimeter precision estimation of population structure, for individuals having a diameter between 5 and 16 cm. This doesn't mean that the detection is not valid for bigger individuals in the site of Ponta Furada, but rather that too few counting data could be collected for the test to be validated above 16 cm. Globally, validation data aggregated from both sites (Fig. 8) confirms the validity of the imagery method for a centimeter precision quantification of population structure, for individuals with diameters above 4 cm diameter, no data being discarded (Chi-square test comparing imagery with all *in situ* validation results: AEF for data between 4 and 34 cm= 531/(2*30)=8.85 > 6; Chi^2= 15.409; dl=29; $p<0.981$).

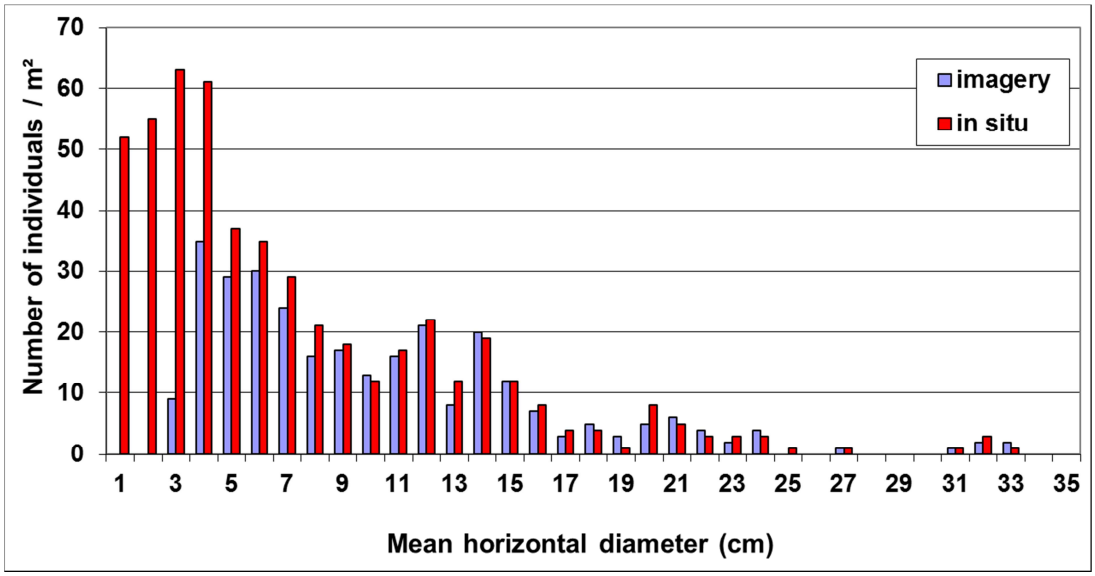


Figure 8 - Size histogram obtained from *in situ* and imagery counting in validation quadrats (data aggregated over time and sites).

It is important to note that we rather consider these conclusions in term of adequacy between two measuring techniques, rather than in term of validation of one (imagery) by the other (*in situ*), as our experience showed that traditional *in situ* counting method is also inevitably affected by its own imprecisions. For instance, for alga above 22 cm diameter, a graduated meter band was used instead of the caliper and underwater perspective effect makes it difficult to localize simultaneously the projection of both edges of the macroalga on the graduated band for a diver who is close to the object; scuba diving difficulties implies the possibility of missing or double counting some of the numerous scattered individuals present; underwater counting's communication between divers, recording and later transcription are subject to mistakes. The remaining inadequacies between the two techniques can result from the following situations. Both results make the model assumptions of "round individuals", whereas they are not; the convention taken to count only the individuals having more than half of their thallus included in the delimited quadrat logically lead to occasional uncorrelated subjective decisions in each approach. During the interval between *in situ* counting and imagery acquisition, some *C. elisabethae* could be detached and carried away by natural hydrodynamics or by occasional unfortunate physical pressures by divers during *in situ* works. This last artifact is underlying yet another advantage of the non-intrusive underwater imagery remote sensing method.

7.3.3 Population dynamics and inter-site differences

7.3.3.1 Evolution of density, mean diameter, cover rate and biomass

In the present study, the imagery approach could produce information over permanent sites totaling 48 m², resolving a seasonal signal of density, biomass and substrate percentage cover (Figs. 9 and 10; details of detection and population histograms in Annexes 10.11 and 10.12). For both sites, these parameters showed a development minimum in the end of winter, a slight increase in spring, clear development in summer and the onset of reduced development in early autumn. Population density showed a sharp reduction in autumn 2003 and did not show a full recovery in spring and summer 2004. Comparing summer 2003 and summer 2004, the proportional reduction of these population parameters was more intense for the site of Caldeirinhas than for the site of Ponta Furada. During the following year (summer 2004 - summer 2005), population of the sheltered site maintained density, while at the exposed site, population density, cover rate and biomass dropped again clearly. For the Ponta Furada population, the higher spring and summer 2005 growth rates (section 3.3.2) could only stabilize temporarily the biomass and cover rate of this population, while density kept on dropping continuously. Over the two years of population monitoring, population of Ponta Furada had shown a mean density reduction from 8 to 1 individual per square meter. It also displayed a general increase of mean diameter (from about 14 to 16 cm diameter). These two changes indicate the proportionally reducing presence of young recruits and small individuals in this population. In the Caldeirinhas, we measured a mean biomass reducing from 730 g dry $wt.m^{-2}$ in the beginning of our monitoring (August 2003), to 300 g dry $wt.m^{-2}$ in the end (November 2005), after a minimum of 243 g dry $wt.m^{-2}$ in March 2005. In Ponta Furada, the mean biomass was already quite lower than in the first site in the beginning of the study: 213 g dry $wt.m^{-2}$ in August 2003. It suffered a more severe proportional reduction than in Caldeirinhas during the monitoring period, reducing to only 34 g dry $wt.m^{-2}$ in November 2005.

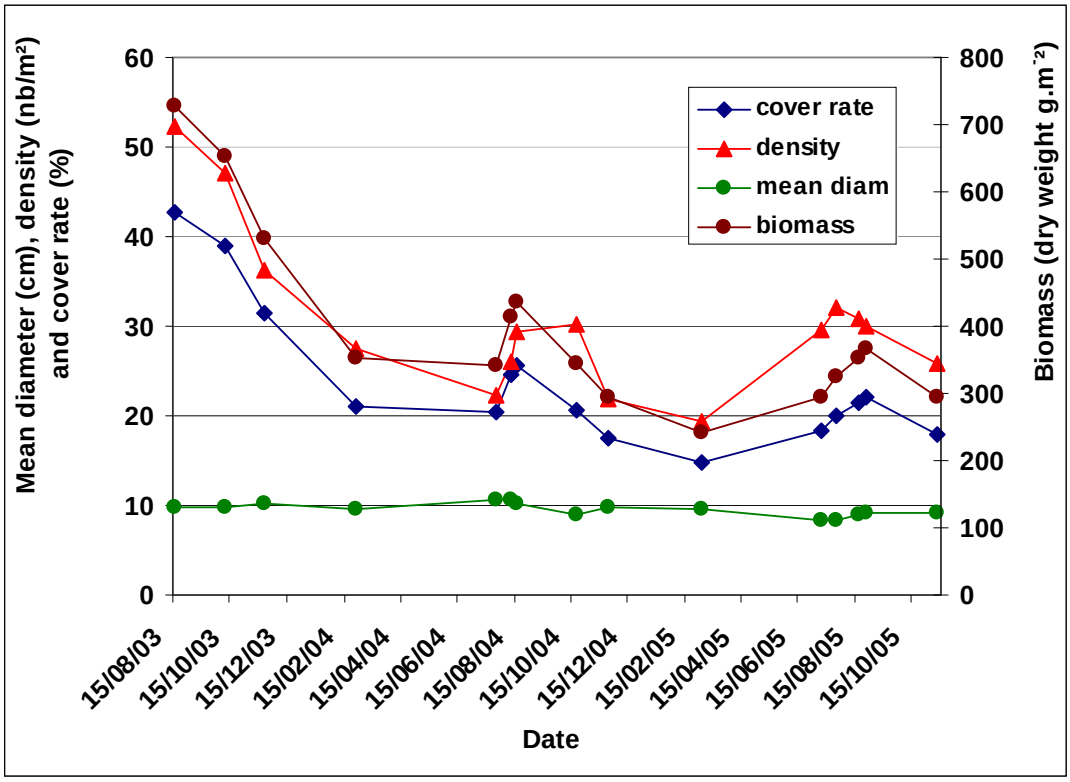


Figure 9 - Evolution of cover rate, density, mean diameter and biomass of *Codium elisabethae* population, site of Caldeirinhas

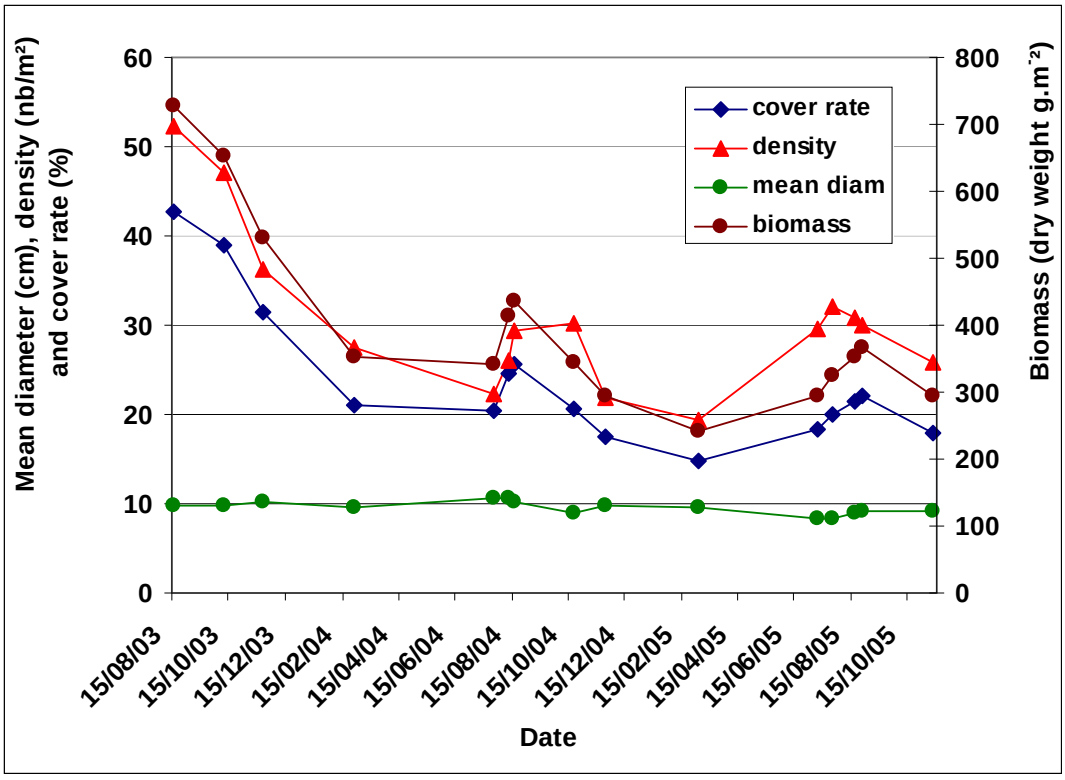


Figure 10 - Evolution of cover rate, density, mean diameter and biomass of *Codium elisabethae* population, site of Ponta Furada

7.3.3.2 Growth rate

Growth rates were never previously estimated for *C. elisabethae*. It was calculated here as the monthly increase of the mean horizontal diameter seen by imagery change detection. Considering time lag between successive image mosaics, all growth detected automatically by the algorithms for persistent individuals are brought to a common temporal basis of one month, before being averaged by period of interest. For this analysis, the limits of the aggregating periods are chosen as follows based on ecological considerations appearing important for algal growth rate within this local coastal environmental dynamics (see Chapter 5). Without referring strictly to the astronomically defined seasons, the following corresponding period abbreviations are used on the figures 11 and 12: ES = End Summer (September - mid October); A = Autumn (mid-October - end December); W = Winter (January, February, March); SP = Spring (April, May, June); S = Summer (July-August). The specific distinction of a late summer – early autumn season (ES) season is based on the clear intermediate situation occurring from September till mid-October in this coastal zone under strong influence of oceanic regime: in this period, the light intensity is starting to reduce, while the seabed ecosystem studied is still under the influence of a warm stratified water column reaching the island coasts from the open ocean. This stratified water regime will remain generally until late in the autumn (till early November, see Chapter 5).
Seasonal fluctuations of growth rate were demonstrated for both sites, with average seasonal values ranging from about 0 cm/month in September- mid-October 2005 in Ponta Furada till about 2.5 cm/month in July-August 2005 in the Caldeirinhas (Fig. 11).

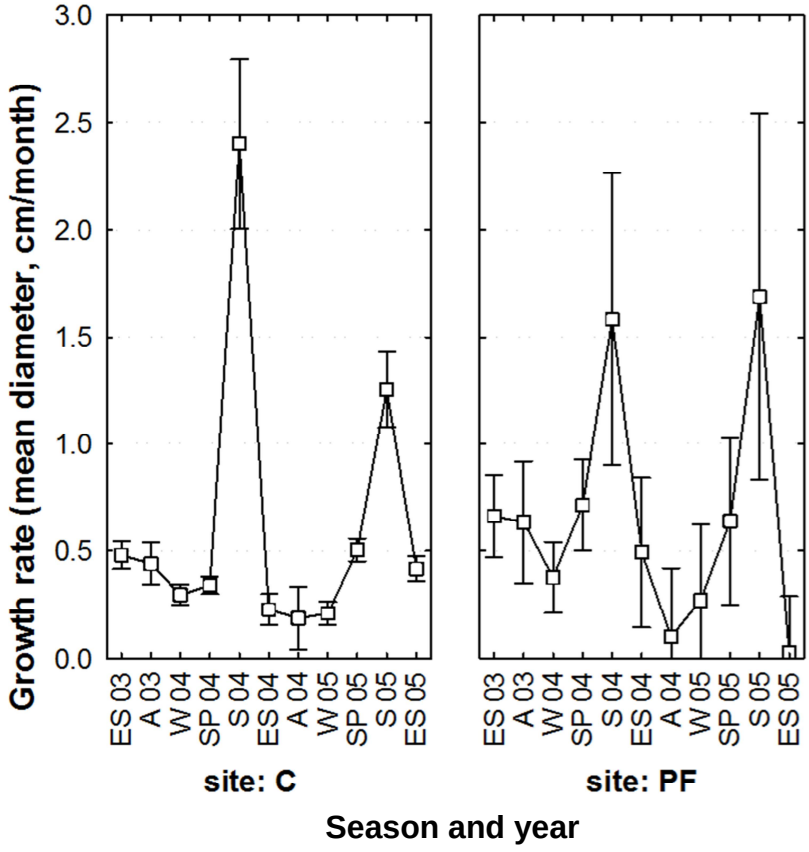


Figure 11 – Seasonal growth rate of both *Codium elisabethae* populations (averaged over size, with confidence interval) during 2 years period; ES = End Summer (September – mid-October); A = Autumn (mid-October - end December); W = Winter (January, February, March); SP = Spring (April, May, June); S = Summer (July-August); 03 =2003; 04=2004; 05=2005.

The statistical analysis of seasonal growth rate averaged over size (Fig. 11) concluded to a significant effect of the factor "season and year" on the growth rate, but no general effect of the site while aggregating all size classes. Nevertheless, mean population growth rate can be significantly different between the two sites for some particular seasons. In these results, the variation of the population structure itself according to site and season might hide some more specific but important effect of the site on growth rate, as a consequence of the non-uniform growth rate over all size classes. Thus, the same analysis performed on particular class sizes might show a significant effect of site on growth rate, but this investigation is left open for further analysis of present data, as further treatment of the unprocessed parts of the imagery database compiled.
Similar analysis is given in figure 12 with a mean growth rate calculated for all data corresponding to the same period independently of the year. It summarizes the global similar mean seasonal cycle of the size-averaged growth rate in each site. Wider confidence intervals displayed in the site of Ponta Furada are mainly justified by the smallest data set due to lower population density. July-August growth rates are clearly higher than during all other seasons. April-June and September-mid-October average growth rates are higher than the mid-October-December and January-March minimal ones. These differences are more pronounced in magnitude in Ponta Furada, although less significant than in Caldeirinhas (probably due to lower sampling effective). Comparatively to Ponta Furada, the lower growth rates measured in Caldeirinhas in September-mid-October and in April-June might be related to the shadowing effect of the particular topography of the Caldeirinhas acting as light limiting factor on growth. Lower temperatures measured in the Caldeirinhas during these periods (see Chapter 5) could also contribute to limit the growth of this population.

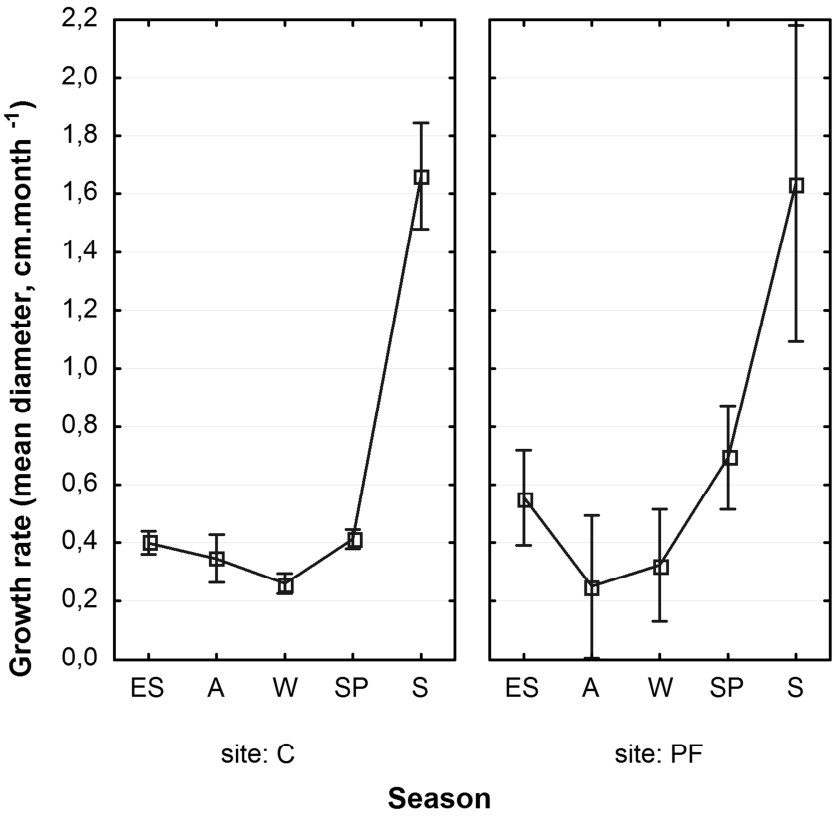


Figure 12 - Seasonal growth rate of both *Codium elisabethae* populations (averaged over size over 2 years); ES = End Summer (September - mid October); A = Autumn (mid-October - end December); W = Winter (January, February, March); SP = Spring (April, May, June); S = Summer (July-August).

Analyzing the global effect of size on growth rate independently from all other variables (Fig. 13), we can see that the mean growth rate obtained for intermediate size class (aggregated as 5-10 cm diameter) is significantly higher than for younger (class 0-5 cm) and older individuals (class 10-15 and 15-20 cm). For size classes above 20 cm diameter, nothing can be affirmed as the confidence interval raises a lot due to the lower representation of these larger individuals in the population. Nevertheless, the apparent second raise of growth rate appearing for larger individuals can be interpreted as consequence of the known modification of shape (flattening of the sphere) occurring for large individuals rather than as pure uniform growth (see Chapter 6). As such, this somehow 'false growth' does not lead to important biomass increase as it is the case for the first pic of growth rate observed during the life time of this macroalga. This phenomenon can also be seen in the slope reduction observed at large diameters in the curve linking biomass and mean horizontal diameter (negative coefficient of higher order of polynomial equation, Fig. 7 in Chapter 6).

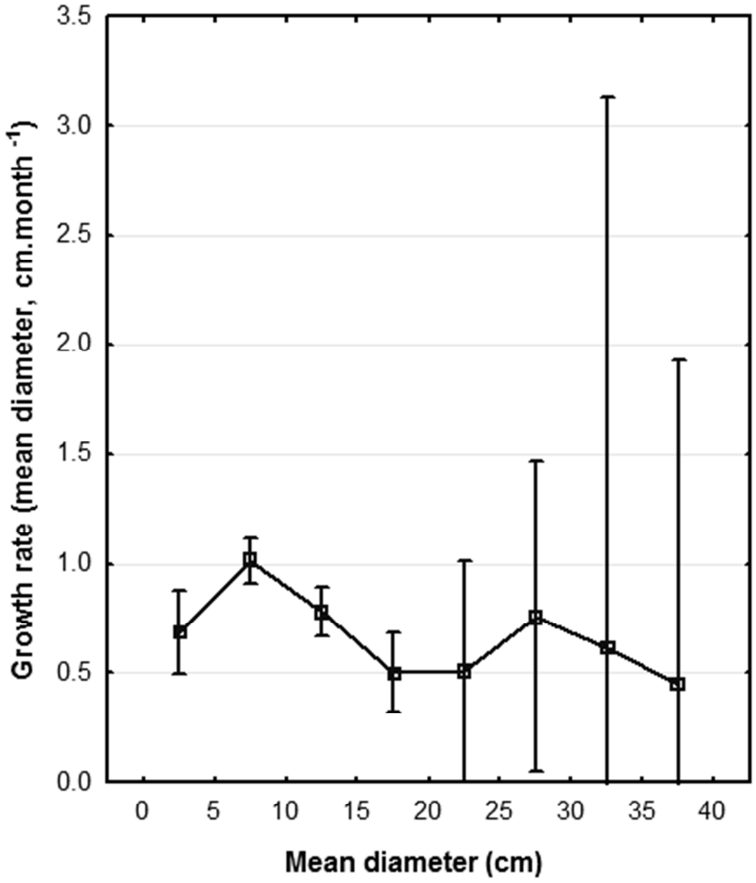


Figure 13 - Seasonal growth rate of both *Codium elisabethae* populations (averaged over both sites and all seasons)

Looking closer at the results from the site of Caldeirinhas only, we can observe that the previously described higher growth rate of intermediate size classes is modulated by the seasons (Figs. 14 and 15), with a strong amplification of that effect in summer, leading to a five times higher growth rate of intermediate size class related to smallest ones (2.5 cm/month for the class 8-10 cm, and 0.5 cm/month for the class 2-4 cm). In winter, the effect of size show similar trends but differences are not significant, and the growth rate values are all very low, ranging from about 0.18 to 0.33 cm/month.

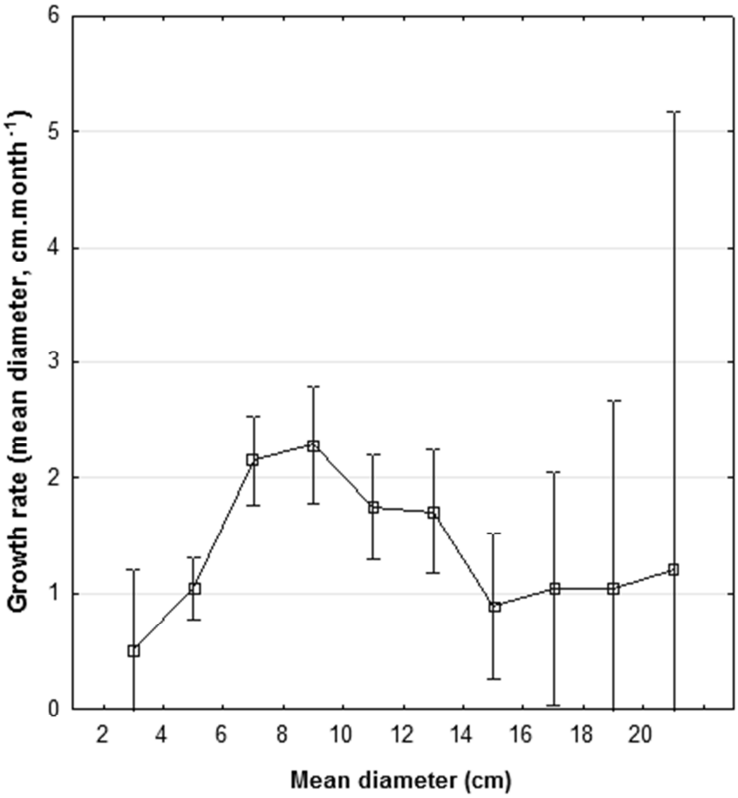


Figure 14 - Mean summer growth rate of both *Codium elisabethae* population for the site of Caldeirinhas.

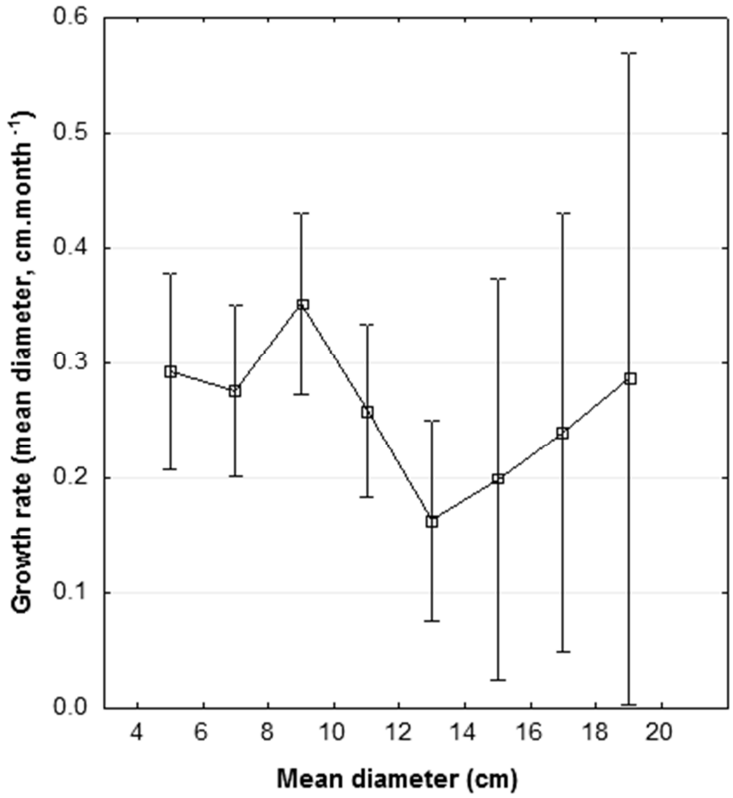


Figure 15 - Mean winter growth rate of both *Codium elisabethae* population for the site of Caldeirinhas.

7.3.3.3 Age to size history and lifespan

Considering only the seasonal modulated values of the mean growth rate of the Faialense *Codium elisabethae* populations, a simple model was used to produce a first estimate of the relation between size and age of the macroalga (Fig. 16). This relation is based on applying our measured growth rate on an initial condition given by a supposed 4 month old individual of 4 cm diameter (extrapolated from the 1cm/month growth rate proposed for youngest *Codium bursa* individuals (Vidondo and Duarte, 1998). On this basis, the model indicates that the largest encountered 50 cm diameter tall *Codium elisabethae* have an approximate age of 7 years. Further refinement of this age to size model will have to into account the combined effect of size and seasons on the growth rate, leading to some potential difference of life growth histories according to season and site of recruitment considered. Another required improvement for this model is the reconsideration or verification of the growth rate of 1 cm/month taken for youngest recruits, as our measurement indicate a positive relation between growth rate and horizontal diameter for sizes comprised between 3 and 9 cm, leading by extrapolation to smaller sizes to growth rate values well inferior to 1cm/month for individuals smaller than 2 cm in diameter. Thus real age of largest individuals could be beyond 7 years.

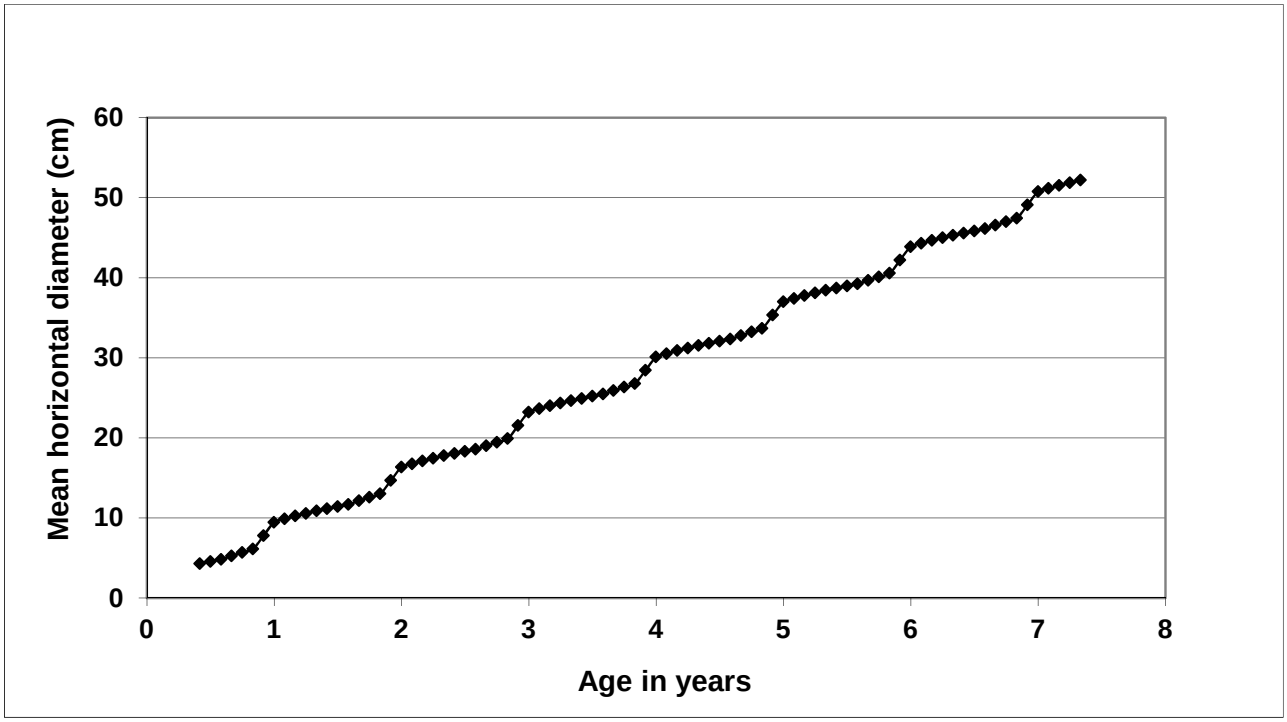


Figure 16 - Mean horizontal diameter of *Codium elisabethae* versus age, simple model considering only seasonal effect on growth rate (for the south coast of Faial island, Azores).

7.3.3.4 Primary production

Over the two years of the monitoring study, the mean values of primary production were evaluated for the first time for *C. elisabethae*, based on population change detection computed from imagery. Values of 2.24 and 1.21 g dry wt.m^{-2}.day^{-1} were measured respectively for the sites of Caldeirinhas and Ponta Furada (Fig. 17). The annual primary production values of *Codium elisabethae* (480-800 g dry wt .m^{-2}.y^{-1}) are significant for the coastal ecosystem. They are for instance comparable to the one measured in the Mediterranean (Calvi bay, Corsica) for the seagrass *Posidonia oceanica* (500-700 g dry wt. m^{-2}.y^{-1}; Champenois, personal communication).

The contribution of recruitment to primary production is relatively more important for the site of Ponta Furada. In Caldeirinhas, the primary production fluctuated from 1.04 g dry wt.m^{-2}.day^{-1} in winter 2005 to 11.5 g dry wt.m^{-2}.day-1 in summer 2004. In Ponta Furada, these values fluctuated between 0.53 g dry wt.m^{-2}.day-1 in spring 2005 and 3.24 g dry wt.m^{-2}.day^{-1} in autumn 2004.

This production is probably under-evaluated as series of frequent *in situ* measurements (couple of days' time lag) had shown a high temporal dynamics on youngest recruits, indicating high rates of advection and predation on the smallest class sizes, which density are underestimated by imagery below 4 cm diameter (Fig. 8).

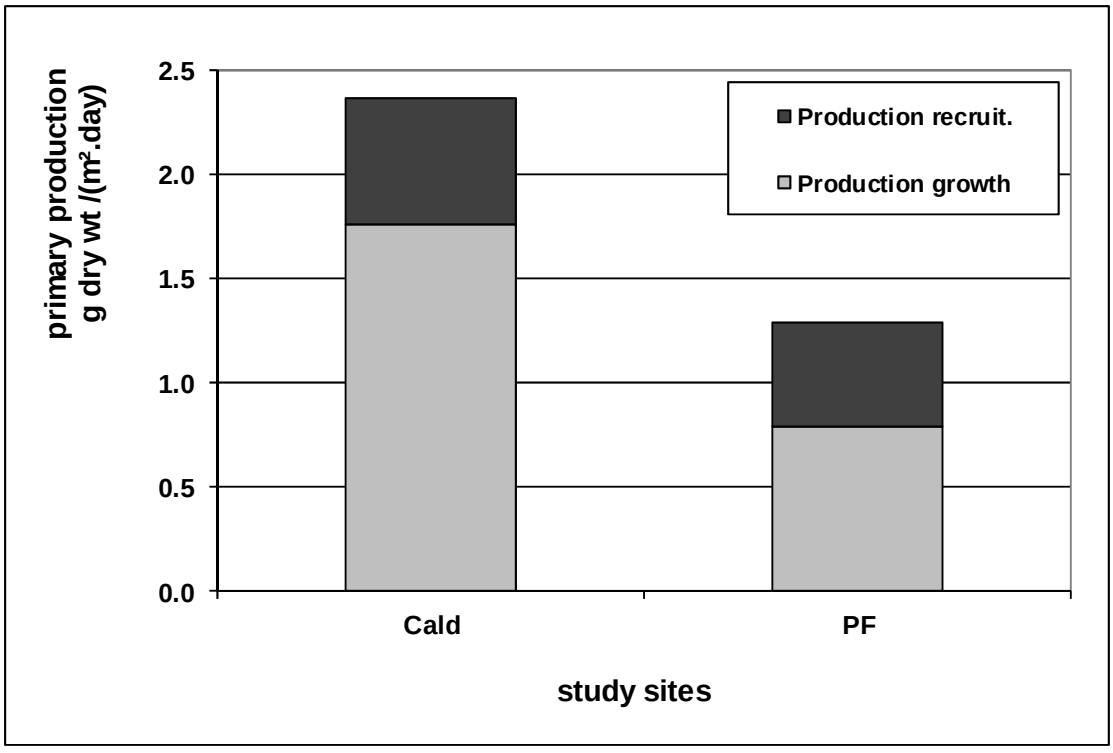


Figure 17 - Total primary production of *Codium elisabethae* populations of the sites of Caldeirinhas (left) and Ponta Furada (right), with distinction of production from growth and from recruitment

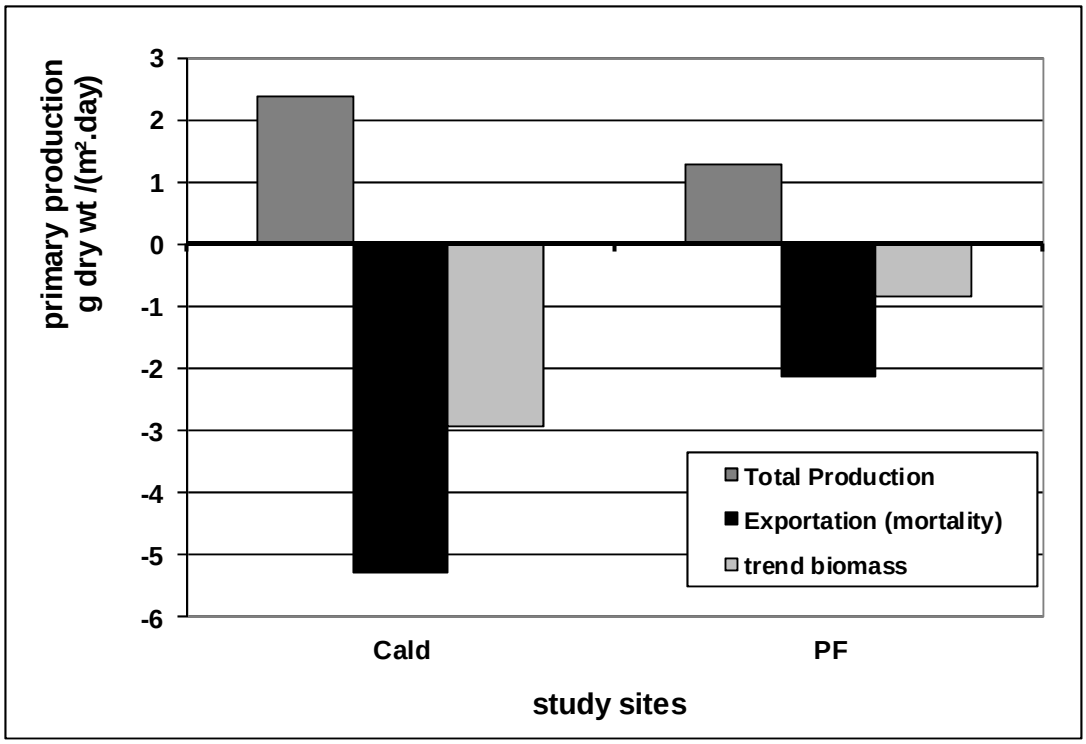


Figure 18 - Overall negative trends of *Codium elisabethae* biomass variation presented as difference between total primary production and total biomass exportation

The general reduction trend of the biomass over the monitoring period (Fig. 18) is resulting from difference between total primary production (due to recruitment and growth) and total biomass exportation. This last term is evaluated by the integration of the biomasses of disappearing individuals. Once detached or broken up with the softer inner part exposed, the decaying or dying individuals were directly observed to be intensely grazed by various benthic herbivorous fishes (and probably crabs and hermit crabs), or to be advected to some lower zones where they accumulate in large amounts (as in the lower zone of the external caldera) and feed the recycling chains (detrital feeders and bacterial decomposition). Occasionally after winter storms, they are massively washed ashore and remain as organic input feeding the sand beach ecosystem associated to marine organic deposits.

7.3.4 Comparison of information produced by imagery with previous knowledge concerning *Codium elisabethae* and *Codium bursa*

The present section brings some of the results produced by visible imagery approach in regards to previously published knowledge about *Codium elisabethae* and its "cousin alike species" *Codium bursa*. Comparisons are made about various population structure and dynamics parameters: density, mean and max diameter, growth rate, life span, biomass, and primary production. Vidondo and Duarte (1998) provided a precise description of a population of *Codium bursa* occupying a 5 to 10 m depth rocky platform in Cala Jonquet, a protected cove in the North-Western Mediterranean Spanish coast. They considered it as a dense population stand, relatively to what is generally encountered for this macroalga. All the

results presented hereafter as comparison about *Codium bursa* comes from studies of this population. The previous seasonal study of *C. elisabethae* population densities (Neto, 1997 and 2000) concerned population occupying sites ranging from 5 to 15 m depth on the north and south coast of Sao-Miguel island (Azores). In the present study, population parameters are produced over constant identical zones of 16 m² and 32 m² according to the site, whereas they were estimated over a random distribution of small quadrats (25x25 cm) totalizing an area fluctuating from 5 to 10 m² according to the density encountered for the study by Vidondo and Duarte (1998), and over a random distribution of small quadrats totalizing a smaller area of 1,8 m² for the study by Neto (2000). These earlier studies constitute a good basis of comparison, but their results might be subject to interference with small scale spatial variability as consequence of the random distribution approaches over such small areas and reduced population samples.

7.3.4.1 Density

Concerning *Codium elisabethae* population on the northern and southern coasts of Sao-Miguel Island (Azores), Neto (1997) mentioned only some trend to higher summer densities, and no seasonality detected concerning the growing cycle. The study by Vidondo and Duarte (1998) mentioned densities of *Codium bursa* fluctuating between 19 to 37 individuals per square meter, with an average over the whole study of 27 individuals/m². Relatively, it can be considered as an intermediate situation between densities of *Codium elisabethae* observed off Faial coast, with less than 1 till 8 ind./m² for the site of Ponta Furada and 20 to 53 ind./m² for the site of Caldeirinhas. While density maximum of *Codium elisabethae* were observed between August and October in our study of Faial populations, the dynamics seems quite different for the *Codium bursa* population in Cala Jonquet, with density minimum in August and two maxima, one in May-June and a more pronounced maximum in November-December, indicating two periods of more intense reproduction and or two periods of enhanced mortality.

7.3.4.2 Mean and maximum horizontal diameter

Studying *Codium elisabethae* populations in Sao-Miguel island, Neto (2000) mentioned observing maximum diameter of 8 cm. Vidondo and Duarte (1998) mentioned a mean diameter varying from about 3 to 6 cm for their study on *Codium bursa*, and maximal observed size of 31 cm. Both of these studies indicates populations constituted of generally smaller individuals than for the populations of *Codium elisabethae* studied in Faial (stable mean diameter of 10 cm in Caldeirinhas and mean diameter fluctuating from 14 to 17 cm in Ponta Furada). The maximal size mentioned in previous studies is rather small as compared to the maximal size of 50 cm observed here in Ponta Furada. This might be due to the shallower habitats investigated by these previous studies (5 to 15 m deep for the work of Neto (2000), 5 to 10 meters for the work of Vidondo and Duarte (1998), comparatively to the present study carried out within the local optimum development depth range (20-25 meters) of *Codium elisabethae* population in Faial-Pico area (Tempera, 2009). Further work would be required to investigate whether this lower maximal size reported by Vidondo and Duarte (1998) and Neto (2000) is resulting from an increased advection pressure in the shallower habitats of these previous studies, from a limitation due to some differences of environmental growth factors (nutrient, light, temperature), biotic factors (epiphytes, symbiosis with specific Cyanophycota), or an auto ecological adaptation.

7.3.4.3 Biomass

Around São Miguel Island, Neto (1997) suggested a stable yearly averaged dry weight biomass of *Codium elisabethae* of 6 g/m². In sub-tidal sites ranging from 5 to 15 m depth on the north and south coast of Sao-Miguel island, Neto (2000) estimated that the yearly averaged dry weight biomass of *Codium elisabethae* was around 6 g/m², and could be considered similar on the northern and southern coasts of this island, as similar between the years 93/94 and 94/95. The *Codium bursa* population examined by Vidondo and Duarte (1998) had an average biomass of 116.4 g dry wt.m^{-2}, and did not show any seasonal pattern. In both sites studied off the south coast of Faial, the biomass of *Codium elisabethae* was high comparatively to the previously mentioned results, with observed ranges of 243 - 730 g dry wt.m^{-2} in the Caldeirinhas, and of 34 - 213 g dry wt.m^{-2} in Ponta Furada. Comparatively to other situation described for *Codium bursa* by Vidondo and Duarte (1998) and for *Codium elisabethae* by Neto (2000), the biomass of *Codium elisabethae* displayed strong seasonal and inter-annual variations.

7.3.4.4 Growth rate

Growth rates were never previously estimated for the species *Codium elisabethae*. Our estimations for the *Codium elisabethae* population present in the southern coast of Faial do reach maximal values clearly superior to the ones measured for *Codium bursa* populations in Spain by Vidondo and Duarte (1995, 1998). These authors mentioned the highest growth rate (1 cm in less than 1 month) for the small recruits (0-2 cm in diameter). Comparatively, our results showed average growth rate of 0.7 cm/month for individuals of size below 5 cm, whereas maximal growth rate is reached later in the life of individuals, when their horizontal diameter is of the order 8 to10 cm, and this growth rate maxima reaches mean values of only 0.3 cm/month in winter but up to 2.5 cm/month in summer. According to Vidondo and Duarte (1998), the specific growth rate of *Codium bursa* is not related to individual diameter under normal oligotrophic conditions, whereas our results showed that absolute growth rates of *Codium elisabethae* in Faial does show a maximum for intermediate size in the summer conditions of this oligotrophic pristine environment (showing concentration ranges of 0.12 – 2.81 µM/l for nitrates, 0.13 – 2.23 µM/l for ammonium and 0.03 - 0.17 µM/l for phosphates, see Chapter 6). If absolute growth rates are shown to be lower for 14-16 cm class size than for the intermediate 6-10 cm class size (Fig. 13), then specific growth rates will also be influenced similarly by individual diameter.

7.3.4.5 Maximum life span

No estimation of *Codium elisabethae* life span could be found up to date in the literature. Geertz-Hansen *et al.* (1994) estimated an age exceeding a decade for the largest *Codium bursa* observed in their study (25 cm), without detailing further the methodology of this estimation. In Vidondo and Duarte (1998), a possible life span exceeding 15 years is mentioned for *Codium bursa*. Our first age to size modelling for *Codium elisabethae* estimated the largest observed individual (horizontal diameter of 50 cm, site of Ponta Furada) to be about 7 years old. Yet accepting the status of long living benthic organism to *Codium elisabethae*, this first estimate would suggest an overall shorter lifespan of that species in the Azorean conditions than for *Codium bursa* in the north-eastern Spanish coast. Most probably this first approach underestimates *Codium elisabethae* lifespan, as it is based on seasonal modulated growth rates averaged over all class sizes, which might result in overestimation of growth rates during the long-lasting large-size life stages in the case of this simple estimation.

7.3.4.6 Primary production

Net primary production was not previously evaluated for *Codium elisabethae.* Vidondo and Duarte (1998) estimated at 0.202 +- 0.05 g dry wt.m^{-2}.day^{-1} the primary production of a high density stand of *Codium bursa* in Cala Jonquet. Comparatively, values of 2.24 and 1.21 g dry wt.m^{-2}.day^{-1} were evaluated respectively for the populations of *Codium elisabethae* studied in the sites of Caldeirinhas and Ponta Furada, which are 6 to 11 fold higher primary productions in average. In Caldeirinhas, the primary production of *Codium elisabethae* fluctuated from 1.04 g dry wt.m^{-2}.day^{-1} in winter 2005 till 11.5 g dry wt.m^{-2}.day^{-1} in summer 2004. In Ponta Furada, these values fluctuated from 0.53 g dry wt.m^{-2}.day^{-1} in spring 2005 to 3.24 g dry wt.m^{-2}.day^{-1} in autumn 2004. Although demonstrations of seasonal variations of macroalgal primary production are available in various environments (Diaz-Pulido and McCook, 2008), the present study quantifies it for the first time concerning Azorean populations of *Codium elisabethae*, and illustrates a new non-destructive approach allowing to access benthic biological data both over large areas while integrating effects of high resolution patchiness dynamic.

7.4 Conclusions

A two-year monitoring of *Codium elisabethae* population structure, growth rate and primary production was conducted in two reference sites selected within the Monte da Guia SAC, Faial, Azores. For this, a rapid and non-invasive underwater imagery methodology was established. Chi-square tests of image-derived estimates and *in situ* measurements confirmed the validity of a centimeter precision estimation of global population structure for individuals above 4 cm in diameter.

The present work demonstrated that density, biomass and substrate cover rate tend to rise in spring and summer, and to drop significantly in end of fall and in winter. Clear seasonality of growth rate was also demonstrated and quantified. Nevertheless, the inter-annual variability of picks of density seems quite pronounced, and a general trend of density reduction was observed since summer 2003 till summer 2005, raising questions on long term trends as on the relative roles of intra/inter-specific factors and of changes in environmental forcing's on these population dynamics. Significant differences of population structure and dynamics parameters are also quantified between the two reference coastal habitats selected. Population density showed a sharp reduction in autumn 2003 and did not show a full recovery in spring and summer 2004. During the following year, population of the protected site maintained density and biomass, while at the exposed site population density dropped. Higher density, cover rate, biomass and primary production were measured in the site of the Caldeirinhas, but higher mean diameter and maximal size were observed for the site of Ponta Furada. These observations suggest a higher success of settling of young recruits in the Caldeirinhas (possible combination of different effects: higher concentration of gametes, lower mean current speed, lower competition for space with other species, lower sand deposit and abrasion effect on early development stages; lower grazing pressure), but a limitation of size development at later stage in the Caldeirinhas (possibly due to intra-specific competition for space or to higher instantaneous current speed due to wave impacts).

The present study quantifies for the first time the seasonal variations of the Azorean *Codium elisabethae* primary production, and illustrates a new non-destructive approach allowing to produce this benthic biological key information over large areas while integrating effects of high resolution patchiness dynamic. In contrast with conclusions of earlier studies on the Azorian *C. elisabethae* (Neto, 1997 and 2000) and on the Mediterranean *C. bursa* (Vaqué *et al.*, 1994; Vidondo and Duarte, 1995, 1998), the present study on *C. elisabethae* populations present in the Monte da Guia SAC revealed higher biomass (34 - 730 g dry wt.m^{-2}), growth rates (up to 2.5 cm/month in summer, that is 3 to 5 fold the previously estimated value for *C. bursa*) and primary production (0.53 – 11.5 g dry wt.m^{-2}.day^{-1}), and demonstrated their clear seasonal and inter-annual fluctuations. *Codium bursa* is presented by Vidondo and Duarte (1995) as a slow growing organism, with population structures suggested being almost constant. Comparatively, the populations of *Codium elisabethae* observed in the Azores are shown to be affected by more important seasonal and inter-annual fluctuations, and their primary production in summer and beginning of autumn can reach very high values, mainly as consequence of intense recruitment of smaller size individuals. Inversely, the dynamic of primary production resulting from the sole growth is clearly less pronounced, from what can be estimated from image analysis. In this sense, our study shows that although the relatively slow growth rate of *Codium elisabethae* and *Codium bursa*, the contribution of these

macroalgae populations to the benthic ecosystem primary production is more consequent than expected when considering only growth rates and global population structure.
The presented imagery-based approach circumvents the need to intensively collect measurements and quantitative information during time-constrained SCUBA diving operations and enables biological population statistics to be robustly derived from observations of thousands of individuals. This technique permitted to acquire exploitable imagery information over a considerably large zone with a limited diving time investment. In addition to produce the first precise quantitative evaluation of the *Codium elisabethae* life cycle and population dynamics, this study demonstrated the potential of simple seabed visible imagery mosaic mapping for detailed long term, frequent, high resolution and spatially continuous quantification of some benthic biological occupation and processes. Together with the coral/algal competition study by Mumby *et al.* (2005), the present study provides one of the first multi-annual monitoring information on the dynamics of a benthic macroalgal population derived from underwater imagery, and the first one to address these issues from metrically corrected continuous image mosaics from which organisms size, population structure, small scale distribution, growth rate, biomass changes and primary production could also be quantified.

This work produced a two year time-series of spatially referenced and geometrically corrected image mosaics which are exploitable for future researches on the spatial organization dynamics of *Codium elisabethae*. Providing the identification of good correlation between *in situ* or laboratory observations and imagery derived parameters, this approach and the available image database may bring new insight for studies concerning various other species occupying the two reference benthic habitats selected within the Azorean SAC of Monte da Guia.

7.5 References

Diaz-Pulido, G. and McCook, L. July 2008. Macroalgae (Seaweeds). In Chin. A, (Ed.) The State of the Great Barrier Reef On-line, Great Barrier Reef Marine Park Authority, Townsville. http://www.gbrmpa.gov.au/corp_site/info_services/publications/sotr/downloads/SORR_Macroalgae.pdf , accessed 30/10/2010.

Garcia-Campos, R., 2001. A proposal to estimate the motion of an underwater vehicle through visual mosaicking. PhD thesis, University of Girona, Spain, 192 pp.

Geertz-Hansen, O., Enriquez, S., Duarte, C.M., Agusti, S., Vaque, D. and Vidondo, B., 1994. Functional implications of the form of *Codium bursa*, a balloon-like Mediterranean macroalga Marine Ecology Progress Series, 108: 153-160. http://www.int-res.com/articles/meps/108/m108p153.pdf

Gracias, N. and Santos-Victor, J., 2000. Underwater video mosaics as visual navigation maps. Computer Vision and Image Understanding, 79(1): 66-91.

ICES, 2005. Report of the Working Group on Habitat Mapping (WGMHM), 5–8 April, Bremerhaven, Germany. ICES CM 2005/E:05 87 pp.

Ifremer, 2010. Logiciel Adelie. http://flotte.ifremer.fr/flotte/Presentation-de-la-flotte/Systemes-sous-marins/ADELIE, accessed 30/10/2010.

Mumby, P.J., Foster, N.L. and Glynn Fahy, E.A., 2005. Patch dynamics of coral reef macroalgae under chronic and acute disturbance. Coral Reefs, 24: 681–692. doi: 10.1007/s00338-005-0058-5

Neto, A.I., 1997. Studies on algal communities of São-Miguel, Azores. PhD thesis in Marine Biology, University de the Azores, Ponta Delgada, 1997.

Neto, A.I., 2000. Observations on the biology and ecology of selected macroalgae from the littoral of São Miguel (Azores). Botanica Marina, 43: 483-498.

Roscoe, J. T. and Byars, J.A., 1971. Sample size restraints commonly imposed on the use of the chi-square statistic. Journal of the American Statistical Association, 66: 755-759.

Salgado, M., 2002. Relatório da bolsa de investigação do projecto MAROV. Relatório interno de Projecto. IMAR - Instituto do Mar, 6 p. http://www.horta.uac.pt/projectos/marov/reports/Final/Salgado_2002.pdf

Salgado, M., Cardigos, F. and Santos R.S., 2001a. Elaboração de mosaicos utilizando o programa Adelie. Relatório interno de projecto. IMAR - Instituto do Mar, 11 pp. http://www.horta.uac.pt/projectos/marov/reports/rel.adelie.pdf

Salgado, M., Cardigos, F. and Santos R.S., 2001b. Medir mosaicos a partir de imagens de vídeo subaquáticas: muitos problemas e algumas soluções. Relatório interno de Projecto. IMAR - Instituto do Mar, 3 pp.

Salgado, M., Cardigos, F. and Santos R. S., 2002. Identificação de *Codium elisabethae*. Relatório interno de Projecto. IMAR - Instituto do Mar, 4p.

Singh, H., Howland, J., Yoerger, D. and Whitcomb, L., 1998. Quantitative photomosaicing of underwater imaging. Proceedings of the OCEANS Conference, September 1988, vol. 1, pp. 263-266.

Sirjacobs, D., 2002. Matlab designed image analysis software for detection of *Codium elisabethae* and calculation of population statistics. Summer training work in the frame of MAROV Project *(*Mapping of Marine Habitats of the Azores using Robotic Ocean Vehicles*)*, co-ordinated by the Department of Oceanography and Fisheries, University of the Azores, Faial Island . ERASMUS grant report. Université de Liège. 31p. + Annexes.
http://www.horta.uac.pt/projectos/marov/reports/Final/Damien_2002.pdf

Sirjacobs, D., Tempera, F., Cardigos, F., Gobert, S., Lepoint, G., Santos, R.S. and Bouquegneau, J.M., 2004a. Study of the structure, distribution and dynamics of *Codium elisabethae* populations in the Azorian reef ecosystem, use of submarine image analysis, in: Mees, J.; Seys, J. (Ed.) (2004a). VLIZ Young Scientists' Day, Brugge, Belgium 5 March 2004: book of abstracts. VLIZ Special Publication, 17: 66-67.

Sirjacobs, D., Tempera, F., Cardigos, F., Gobert, S., Lepoint, G., Santos, R.S. and Bouquegneau, J.M., 2004b. Study of the structure, distribution and dynamics of *Codium elisabethae* populations in the reef ecosystem of Faial Island (Azores), use of submarine image analysis, in: Proceedings of the 5th International Symposium on Fauna & Flora of Atlantic Islands, Dublin (Ireland), 24-27/08/04.

Sirjacobs, D., Tempera, F., Cardigos, F., Gobert, S., Lepoint, G., Santos, R.S., and Bouquegneau, J.M., 2006. Study of the structure, distribution and dynamics of *Codium elisabethae* populations in the reef ecosystem of Faial Island (Azores), use of submarine image analysis, in: Mees, J.; Seys, J. (Ed.) (2006). VLIZ Young Scientists' Day, Brugge, Belgium 31 March 2006: book of abstracts. VLIZ Special Publication, 30: 56.

Tempera, F., 2009. Benthic habitats of the extended Faial Island shelf and their relationship to geologic, oceanographic and infralittoral biologic features. PhD thesis, 23-06-09, University of St. Andrews, UK, 321 pp. http://hdl.handle.net/10023/726

Vaqué, D., Agusti, S., Duarte, C.M., Enriquez, S. and Geertz-Hansen, O., 1994. Microbial heterotrophs within *Codium bursa*: a naturally isolated microbial food web. Marine Ecology Progress Series, 109: 275-282.

Vidondo, B. and C.M. Duarte, 1995. Seasonal growth of *Codium bursa*, a slow growing Mediterranean macroalga: *in situ* experimental evidence of nutrient limitation. Marine Ecology Progress Series, 123: 185-191.

Vidondo, B. and C.M. Duarte, 1998. Population structure, dynamics and production of the Mediterranean macroalga *Codium bursa* (chlorophyceae). Journal of Phycology, 34(6): 918-924. doi: 10.1046/j.1529-8817.1998.340918.x

Youssef, W.B.H., 2005. Caractérisation *in situ* de l'environnement physique des habitats benthiques occupés par *Codium elisabethae* au sein de sites d'études particuliers (Faial, Açores). Mémoire de D.E.A en Océanologie. Université de Liège, Liège. 94 pp.

Zar, J.H., 1999. Biostatistical Analysis. 4th Edition. Prentice-Hall, Inc., NJ, 931 pp.

8 - General conclusions

Table of contents

8.1 Summary of new knowledge concerning *Codium elisabethae* and the selected seabed habitats of Monte da Guia SAC

The present work contributions can be summarized by the following results:

- **The physical and trophic environmental conditions affecting benthic organisms are precisely quantified for the two 20 meter deep habitats selected as reference study sites within the Monte da Guia SAC:**

  - Current regime is mainly defined by typical East-West tidal currents superimposed on the general Azores Current in the Ponta Furada site. The site of the Caldeirinhas is sheltered from this main coastal current and its complex local regime results from tidal inflow and outflow into the caldera's basins and from wave and wind induced return circulation displaying a dominant north-south component. Twenty minutes average current speed are generally lower in the sheltered Caldeirinhas, except on the occasion of intense southern wind and wave forcing (up to 0.6 m/s). Maximum instantaneous speeds (2 m/s) affecting these 20m deep habitats do correspond to intense swell impact (significant wave height Hs of 5 m).
  - The instantaneous values of temperature range from 16°C in March till 23 °C in September. The monthly mean temperature values tend to be lower in the Caldeirinhas than at the Ponta Furada site throughout the year, with a difference ranging from 0.1°C during the period December-March up to 1°C in the period August-October. A thermal oscillation in phase with the tidal signal can be detected during the stratification period which affects these benthic sites from mid-May to early November. Various observations reveal that the Caldeirinhas site is frequently completely occupied by a massive input of “below thermocline” colder waters during the stratified periods, and is generally more influenced by deeper waters than the Ponta Furada site.
  - The turbidity is much higher in Ponta Furada (range of 1 to 100 NTU) than in the Caldeirinhas (below 1 NTU) presumably due to the extensive sand fields present in the vicinity of the Ponta Furada reef site.
  - The monthly mean daily photon fluxes reaching these benthic habitat ranges globally from 1 to 7 $mol.m^{-2}.d^{-1}$ and are higher at the Ponta Furada site than in the Caldeirinhas, both in winter and in summer. The duration of non-null PAR signal is shorter in the Caldeirinhas habitat as compared to the Ponta Furada site (from 20 minutes in summer up to 2 hours in winter). These differences are due to the high crater slopes surrounding the Caldeirinhas seabed habitat.

- Coastal waters in the south of Faial can be considered as an unperturbed oligotrophic environment with concentration ranges of 0.12 – 2.81 µM/l for nitrates, 0.13 – 2.23 µM/l for ammonium and 0.03 – 0.17 µM/l for phosphates. In winter, no significant differences in nutrient concentration could be detected between both sites. A summer nutrient depletion was demonstrated and lead to lower nutrient concentrations in the site of Ponta Furada when compared to Caldeirinhas, with significant differences between sites for nitrate and phosphate. Considering each site separately, the summer nutrient depletion is significant for the nitrates and phosphates in Ponta Furada, whereas in the Caldeirinhas they are maintained at relatively high concentrations in summer but with significant (summer) depletion in ammonium. These differences are explained by the more important influence of below thermocline nitrate/phosphate rich waters in the site of Caldeirinhas, and possibly by a more important exposition of the site of Ponta Furada to diffused natural runoff and sewage discharges.
- The average salinity observed in the Caldeirinhas was of 35.9 with frequent intense salinity drop events observed in conjunction with important rain events.

Laboratory studies on 200 *Codium elisabethae* allowed for the first time to :

- **Establish a satisfying model (r^2=0.932) expressing thallus biomass versus mean horizontal diameter**, hence allowing integrated biomass evaluations by imagery.
- **Demonstrate the important global nutrient concentration ratio existing between the lumen water of *Codium elisabethae* populations and their surrounding sea water (NO3: 5.7; NH4: 3.4; PO4: 3.1),** as previously demonstrated for *Codium bursa* in the Mediterranean Sea (Geertz-Hansen *et al.*, 1994; Vidondo and Duarte, 1995). These global concentration ratios are higher for the populations of Ponta Furada than for the ones of Caldeirinhas. In the Ponta Furada population, all summer concentrations are significantly lower than in winter, whereas in the Caldeirinhas population the summer concentrations are only slightly lower than winter ones, and the difference is only barely significant for the nitrate. In summer, internal nutrients concentrations are non-significantly different between the populations of both sites. In winter, all internal water nutrients concentrations tend to be superior for the population of Ponta Furada, but the difference is only significant for ammonium.
- **Quantify the seasonal dynamic of the sexual reproduction of *C. elisabethae*, and demonstrate the persistent summer fertility**. The density of utricles with gametangia and the proportion of gametangia still carrying gametes are higher in December than in February and summer periods. In summer, the reproduction is less intense than in winter, but it is not null. Density of utricles is globally significantly increasing with mean horizontal diameter for all periods tested. Maximum densities of gametangia are reached for a mean horizontal diameter ranging from 10 to 18 cm. Generally, sexual reproduction tends to be more precocial and intense for the population of the Caldeirinhas, comparatively to that of Ponta Furada.

- **Quantify the vegetative reproduction during the annual cycle.** For both sites, the total number of propagules per alga did not appear to be significantly affected by the seasonal timing of observation. For each collecting period, it was significantly more intense for the population of the Caldeirinhas, with frequent parent plant carrying 10 to 60 propagules (with a peak record reaching 167 propagules on a single plant) comparatively to maxima of 8 propagules per plant observed in Ponta Furada. The diameter of parent algae was shown to have a significant effect on the total number of propagules carried, with two phases of more intense vegetative reproduction during the algae life. A propagule production phase for parent plants between 6 and 9 cm in the Caldeirinhas and between 6 and 11 cm in Ponta Furada was first observed, followed by a growth period with almost no propagule production (from 9.5 till 11.5 cm in the Caldeirinhas and from 11 till 15 cm in Ponta Furada), and then with a second vegetative reproduction phase again apparent.
- **Quantify global epiphyte biomass on *Codium elisabethae.*** These values increase globally with their age and diameter, being higher in summer than in winter for those individuals larger than 12 cm. The maximal values of epiphyte biomass are found in Ponta Furada for both summer and winter month periods.

⅄ ***In situ* counting's and size measurements allowed us to :**

- **Quantify the general differences of population density, size structure, recruitment intensity, and patchiness between both study sites.** Higher density and more continuous size distribution for Caldeirinhas population were found, whereas bigger individuals (diameter > 15 cm) were more frequent in the site of Ponta Furada, and reached larger maximal size than in the Caldeirinhas (32 cm in the latter versus 24 cm in the former). An important spatial variability of structures and densities at a small spatial scale (1m) was demonstrated within each of the two sites, both during summer and winter periods. Mean density of young recruits produced by sexual reproduction (diameter < 2 cm) was clearly higher in the site of the Caldeirinhas (order of 20 ind./m²) than in Ponta Furada (order of 1/m²), for both summer and winter. The low density of recruitment remained almost stable in Ponta Furada, whereas more intense spatio-temporal dynamics were observed in Caldeirinhas.
- **Validate the centimeter precision estimation of *Codium elisabethae* population size structure derived by imagery methodology for individuals above 4 cm in diameter.**

- **The fluctuations of density, biomass, population structure, growth rate, and primary production of the studied *Codium elisabethae* populations were quantified over a 2 year period based on the non-invasive seabed imagery methodology established. The main results of this study are:**

  - Significant differences of population structure and dynamics parameters between the two reference coastal habitats: higher density, cover rate, biomass and primary production were measured in the site of Caldeirinhas, but higher mean diameter and maximal size were observed for the site of Ponta Furada.
  - Confirmation and quantification (based on a large data sample) of the global tendency of density, biomass and substrate cover rate to raise in spring and summer, and to drop significantly towards the end of fall and in winter, as previously suggested by Neto (1997 and 2000) from random observations on shallower populations off São-Miguel island.
  - A general trend of density reduction measured from summer 2003 till summer 2005. Population density showed a sharp reduction in autumn 2003 and did not show a full recovery in spring and summer 2004. During the following year, population of the protected site maintained a stable density and biomass, while at the exposed site the population density dropped significantly.
  - In contrast with conclusions of earlier studies on the Azorean *C. elisabethae* (Neto, 1997 and 2000) and on the Mediterranean *C. bursa* (Vaqué *et al.*, 1994; Vidondo and Duarte, 1995 and 1998), the present study on *C. elisabethae* populations revealed higher biomass (34 - 730 g dry wt.m^{-2}), growth rates (up to 2.5 cm/month in summer, that is 3 to 5 fold the previously estimated value for *C. bursa*) and primary production (0.53 – 11.5 g dry wt.m^{-2}.day^{-1}), and showed both seasonal and inter-annual fluctuations.
  - First age to size modelling for *Codium elisabethae* estimated the largest observed individual (horizontal diameter of 50 cm, site of Ponta Furada) to be about 7 years old. Yet accepting the status of long living benthic organism to *Codium elisabethae*, this first estimate would suggest an overall shorter lifespan of that species in the Azorean environment when compared to the 10-15 years estimates for *Codium bursa* in the north-eastern Spanish coast (Geertz-Hansen *et al.*, 1994; Vidondo and Duarte, 1998).
  - Comparatively with *Codium bursa* presented by Vidondo and Duarte (1995) as a slow growing organism with almost constant population structures, the populations of *Codium elisabethae* observed in the Azores are shown to be affected by more important seasonal and inter-annual fluctuations, and their primary production in summer and beginning of autumn can reach very high values, mainly as consequence of intense recruitment of smaller size individuals. Inversely, the dynamic of primary production resulting from the sole growth is clearly less pronounced than the one resulting from recruitment from what can be detected from image analysis.
  - In this sense, our study highlights that despite the relatively slow growth rate of *Codium elisabethae* and *Codium bursa*, the contribution of these macroalgae populations to the benthic ecosystem primary production can be more important than expected when considering solely growth rates or population structure.

8.2 Summary of interpretations and hypothesis concerning the observed populations structures and dynamics

This section summarizes the most interesting observations inferred from the comparison between both studied populations. The possible links between the various information produced (benthic habitat environment – reproduction – autecology – population structures and dynamic) are also summarized as a qualitative orientation preparing future detailed multivariate analysis of the whole dataset and as a basis for the identification of complementary data as required.

Difference of populations' structures and evolution between sites
The higher density, cover rate, biomass and primary production for the site of the Caldeirinhas suggest a higher colonization success of young recruits in the Caldeirinhas, possibly as a combination of different effects such as higher production of gametes and propagules, lower mean current speed, lower competition for space with other species, and lower sand deposit and abrasion effect on early development stages. The higher mean population diameter and maximal size observed for the site of Ponta Furada suggest a limitation of size development at later stage in the Caldeirinhas, possibly due to intra-specific competition for space or to higher instantaneous current speed explained by the occasional amplification of wave impacts due to local topography.

Differences of nutrient availability in surrounding seawater
The availability in sea water nutrients is higher in the Caldeirinhas sites in summer, when light and temperature can allow more intense growth rates. This higher seawater nutrient availability in the Caldeirinhas might result in a higher remaining nutrients availability in the internal water (or better physiological status) in the fall, allowing better resources investment for the most intense phase of sexual reproduction occurring mainly under fall/winter conditions.

Differences of lumen nutrient concentrations
The lower summer nutrient depletions observed in the lumen water of Caldeirinhas population (mainly for phosphates) could favor faster growth compared to populations of Ponta Furada. This result is interesting in the perspective of phosphate concentration and was previously proposed as a limiting growth factor for *Codium bursa* populations by Vidondo and Duarte (1995). An interesting trend observed is that of internal nutrient concentrations which tend to be maximum in February and minimum in July-August for both sites. In addition, in December the concentrations are still at a low level in the Caldeirinhas population, while they have already started to rise for the population of Ponta Furada. Further sampling and investigations are required to confirm this trend but we suggest that in December, a more intense sexual reproduction in the Caldeirinhas would still contribute to consume internal nutrients preventing their accumulation.

Differences of lumen nutrient concentration ratios vs seawater
Considering the global trend to lower concentrations in Ponta Furada seawater, the higher absolute lumen nutrients concentrations and global concentration ratios measured in the population of this site constitute an additional argument to understand the particular shape of these macroalga as an efficient functional adaptation to oligotrophic conditions.

Differences of PAR reaching habitats

Global differences of PAR reaching habitats and of temperature would be expected to favor growth rates of the Ponta Furada population, but such difference was not apparent during the analysis. This would suggest that the growth of these low compensation point macroalga (able to live down to more than 40 m) is rather nutrient-limited. Furthermore, the higher PAR detected in Ponta Furada might justify the higher epiphytism observed, which, in turn, prevents *Codium* populations to benefit from enhanced light conditions. A known trigger of sexual reproduction (reduction of light duration) might be activated sooner in the season in the site of the Caldeirinhas due to local shadowing effects of the surrounding topography (as measured by shorter non null PAR recorded at seabed). An earlier on-set of the enhanced fall/winter phase of gamete production might highly benefit the level of gamete production for the Caldeirinhas population, considering the more favorable temperature and light conditions, as compared to a reproduction investment occurring later in the season. More gametangia counting would be required during the August-December period to investigate such effect.

Seasonal description of marine dynamics and growth rate of *Codium elisabethae*

It is interesting and comforting to note the adequate correspondence between the period limits selected by (1) physical oceanographers for the description of seasonal changes of the Azores current and SST patterns and (2) the present study for the description of seasonal changes of growth rate of the long living macroalga *Codium elisabethae*. The periods chosen for large scale oceanographic descriptions are Jan-Feb-Mar; Apr-May-Jun; Jul-Aug-Sep and Oct-Nov-Dec (Mariano, 2011), which are identical limits as used for the growth rate analyzed by imagery (see chapter 7), with the exception of the additional intermediate period lasting from September to mid-October for which important growth factors do justify a distinct analysis from a coastal ecological point of view. To a certain extent, this correspondence confirms that the growth rate of *C. elisabethae* population installed at their optimal depth range (corresponding to the depth of maximal seasonal thermocline gradients), do reflect well the periods chosen by physical ocean scientists to describe the dynamics of large scale features as the Azores current.

Difference of vegetative reproduction intensity or dynamics

The limited size of propagules present on the Ponta Furada population suggests that more intense hydrodynamic or sand abrasive pressures might limit their period of growth on the parent alga. The globally lower amount of propagules detected for the Ponta Furada population might result from the same factors reducing their lifetime as attached, but can as well result from reduced propagule production due to internal limiting physiological conditions (growth, population density). The final fate of these propagules is still unknown. Possible scenarii suggest that 1) some might remain attached to the parent plant as a substrate until decomposition of the latter, or 2) might be detached from the parent plant under the action of hydrodynamic forces with subsequent fixation to the substrate and growing, or 3) might be advected away, with subsequent death and decomposition. The actual likelihood and contribution of these suggested pathways remain to be verified and quantified in order to fully allow accurate population dynamic modelling and understanding.

Differences of young recruit settlement densities, dynamics and patchiness

The higher density of very young recruits in the Caldeirinhas can be explained both by the higher reproduction investment observed for that population, and by a probable higher installment success of larval stages (lower proportional dispersion out of the population stand due to reduced global advection, higher sheltering within a more important bottom limit hydrodynamic layer created by more dense installed population, and lower sand abrasion).

The low density of *in situ* recruitment remained almost constant in Ponta Furada, whereas more intense spatio-temporal dynamics were observed in Caldeirinhas. This fact, together with the intense small scale patchiness of young recruits observed in this site, can be explained by the random localized grazing pressure of benthic herbivorous fish schools. Such feeding behavior was frequently observed in the study sites, and its small scale spatially structuring impact on the Caldeirinhas *Codium elisabethae* population might be enhanced due to its largely dominant biomass and lower epiphytic biomass, probably allowing in turn a more selective feeding attitude towards these young macroalga. Moreover, herbivorous fish populations are most probably denser in the Caldeirinhas since the benthic primary production is higher (at least concerning *Codium elisabethae)*, the reef habitats offer naturally more shelters, and the site is a 'no go' reserve.

Difference of population morphology between sites

The flattening shape evolution is shown to be more pronounced for the Ponta Furada population. This is probably a consequence of lower intraspecific competition for space or of a naturally enhanced elimination of highest individuals by advection due to higher average current speed at this site.

Suggestion of life history synthesis for *Codium elisabethae* (autecology; Fig. 1):

- phase 1: settling of recruits by fixation of the rhizoidal filament and growth
- phase 2: 6 to 10 cm diameter: phase of maximal growth rate to compete for space and light access and reach sufficient diameter to escape grazing pressure. This phase corresponds to the period of maximum nutrient concentration in the lumen. During this phase, the maximal density of gametangia (sexual reproduction) is attained, and remains rather high until the algae's diameter reaches 15-20 cm. A first but moderate maximum of vegetative reproduction is also reached during this phase.
- phase 3: 10-14 cm diameter: the minimum internal nutrient concentrations values are observed and the growth rate is progressively reducing. Sexual reproduction (density of gametangia) remains as intense as found during phase 2, whereas vegetative reproduction is almost absent.
- phase 4: 15-20 cm diameter: growth is at its minimum value, vegetative reproduction is at maximal intensity, and internal nutrient is slightly raised comparatively to previous phase, but remains lower than in phase 2. Sexual reproduction remains at a high level in Ponta Furada, but is reduced in the Caldeirinhas.
- phase 5: above 20 cm diameter, internal nutrients and reproduction reduces, while a phase of apparent raising growth rate is observed, probably due to a phase of shape change corresponding to general flattening and increased central flattening.
- phase 6: death by hydrodynamic impact or decomposition when increasing shadowing by epiphytes prevents photosynthesis to satisfy global respiration requirements. Once the thallus are detached or opened, thus exposing the soft inner side (no utricles), they are intensely grazed by herbivorous.

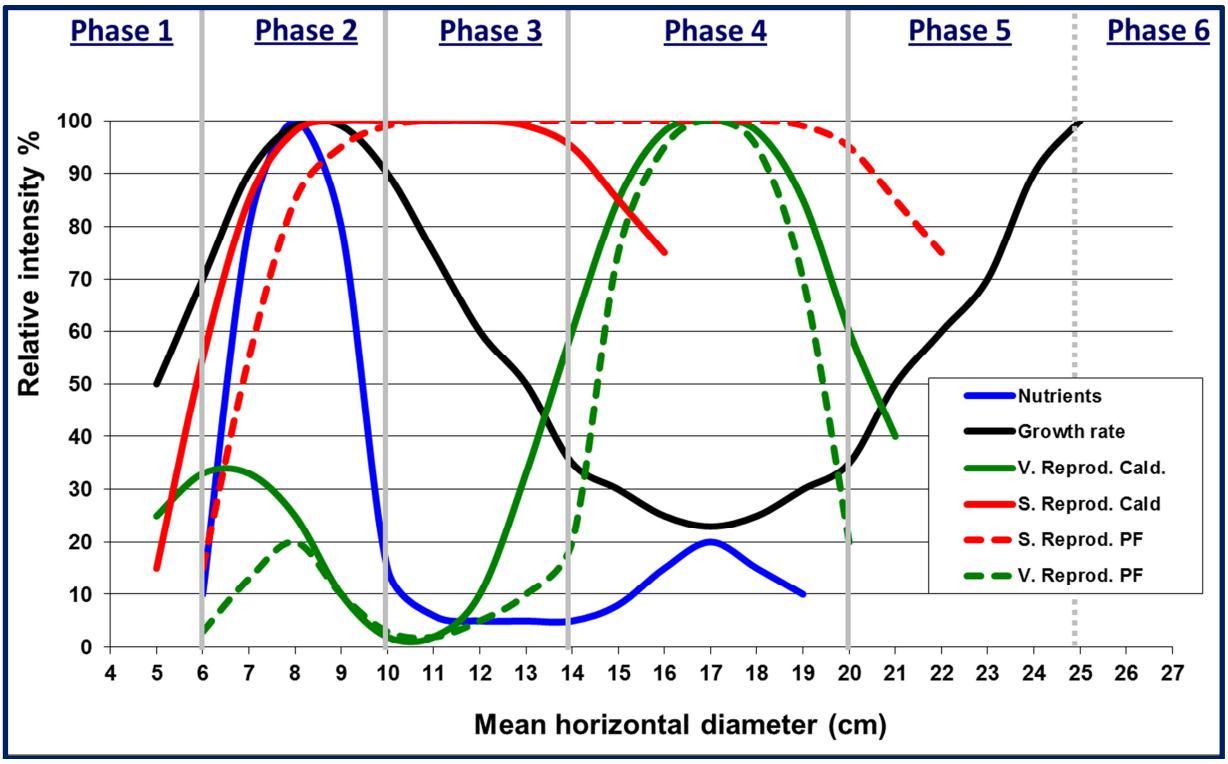


Figure 1 – *Codium elisabethae* autecology synthetic life history phases proposed; based on the evolution of relative intensity (% of maximal values) of important parameters derived from imagery analysis and *in situ* measurements: global mean nutrient concentration in the lumen (blue) and growth rate (black); vegetative (green) and sexual reproduction (red) intensities for both study sites (full lines for Caldeirinhas and dashed lines for Ponta Furada).

8.3 Suggestions concerning potential use of *Codium elisabethae* populations as bio-indicators of environmental changes

Considering the confirmation of similar properties between *Codium elisabethae* and *Codium bursa* (long living sessile organism, nutrient concentration enhancement in lumen, probable nutrient limited growth in normal oligotrophic conditions), we can expand the following suggestion of Duarte (1999) to the *Codium elisabethae* macroalga: « the long living organisms macroalga *Codium bursa*, the seagrass *Posidonia oceanica* and the bivalve *Pinna nobilis* could provide an insight into the ecosystem responses to environmental changes if they could be appropriately interrogated ».

Concerning this 'interrogation', it seems that **3 types of environmental information's could potentially be derived from observations on *Codium elisabethae* populations, providing that further specific research are carried out**:

(1) **Mean coastal seawater nutrient concentration** determination is a complex exercise considering its highly variable nature in the coastal environment, particularly close to seabed (transitory interaction effects of stratification and tide, passing currents or internal wave, bottom sediment re-suspension). Unless based on very frequent measures, evaluations of nutrient concentrations for a typical coast or period are typically biased by some subsampling on a highly variable signal, whereas long living benthic macroalga do integrate the high temporal variability of this important growth factor into one or more potentially measurable parameters. Providing various pre-requisites are satisfied (i.e. these organisms growth would be in nutrient limiting growth condition, or their supporting light and temperature conditions are measured), **the growth rate or internal lumen nutrient concentration of particular class size could be tested as a proxy of the integrated average nutrient concentrations in the surrounding water**. In the same way that chalk plates erosion are used as a proxy for the evaluation of integrated current and turbulence over particular periods.

(2) Slight changes of benthic physical environmental factors (water column stratification regime, light, current, waves) should affect this macroalga growth, reproduction and 'mortality' resulting in final changes in global population structure or dynamics, efficiently accessible over large scale by imagery approach. Providing a good knowledge of the local natural seasonal dynamic and the monitoring of some complementary environmental factors, **changes in population parameters could be tested as a proxy to occurring long term changes in local hydrology.**

(3) **Changes in the large scale biogeographic population distributions** may also be interpreted in terms of **changes in seasonal climatology of water masses at the basin scale**.

Concerning these 3 points, the studies initiated in the Spanish Mediterranean coast in 1994 (Geertz-Hansen *et al.*, 1994; Vidondo and Duarte, 1995) and in the Azores in 2003 (present research) would provide a first valuable baseline for long term monitoring and a robust basis for comparisons with other reference sites.

8.4 Final conclusions

- Two reference study sites were selected for long term monitoring of macroalgae population within the Special Area of Conservation of Monte da Guia, Faial, Azores. They are identified with reference marks on the rocks and equipped with docking stations for complete battery of sensors.
- Seasonal environmental conditions experimented in these sites by benthic organisms are extensively quantified and interpretations of local hydrodynamic patterns driving them are proposed.
- Dynamics of reproduction and of internal nutrient concentration factors of *Codium elisabethae* populations are quantified.
- A 2 year long database of high resolution geometrically corrected seabed mosaics imagery is compiled for both reference sites. The resolution and spectral information available in the imagery database acquired closer to seabed coverage allow foreseeing complementary studies on the ecology and dynamics of *Codium elisabethae,* as of other benthic organisms including the macroalgae *Zonaria tornefortii,* crustose *Corallinaceae, Padina pavonica.*
- Population structures, dynamics (growth rates and primary production) of *Codium elisabethae* are precisely described from *in situ* observations and imagery analysis, with demonstrated seasonal signals. Integrated interpretations of many of the site specific differences are given.
- The new information produced is compared to pre-existing knowledge established in Sao Miguel island (Azores) for *Codium elisabethae* and in Cala Jonquet (Spain) for *Codium bursa,* and the important parameters qualifying *Codium bursa* as potential bioindicator of environmental changes are extended to *Codium elisabethae.*
- The potential of underwater imagery as an efficient tool for expanding the possibilities of benthic habitat and ecology studies is demonstrated, as for the increasing spatio-temporal scales accessible for monitoring.

8.5 References

Duarte, C.M., Agustí, S., Kennedy, H. and Vaqué, D., 1999. The Mediterranean climate as a template for the Mediterranean marine ecosystem: The example of the NE Spanish littoral. Progress in Oceanography, 44: 245-270.

Geertz-Hansen, O., Enriquez, S., Duarte, C. M., Agusti, S., Vaque, D. and Vidondo, B., 1994. Functional implications of the form of *Codium bursa*, a balloon-like Mediterranean macroalga. Marine Ecology Progress Series, 108: 153-160.
http://www.int-res.com/articles/meps/108/m108p153.pdf

Mariano, A.J., 2011. http://oceancurrents.rsmas.miami.edu/atlantic/azores_3.html, accessed 15/08/11.

Neto, A.I., 1997. Studies on algal communities of São Miguel, Azores, PhD thesis in Marine Biology, University de the Azores, Ponta Delgada.

Neto, A.I., 2000. Observations on the biology and ecology of selected macroalgae from the littoral of São-Miguel (Azores). Botanica Marina, 43: 483-498.

Vaqué, D., Agusti, S., Duarte, C. M., Enriquez, S. and Geertz-Hansen, O., 1994. Microbial heterotrophs within *Codium bursa*: a naturally isolated microbial food web. Marine Ecology Progress Series, 109: 275-282.

Vidondo, B. and Duarte, C.M., 1995. Seasonal growth of *Codium bursa*, a slow growing Mediterranean macroalga: *in situ* experimental evidence of nutrient limitation. Marine Ecology Progress Series, 123: 185-191.

Vidondo, B. and Duarte, C.M., 1998. Population structure, dynamics and production of the Mediterranean macroalga *Codium bursa* (*Chlorophyceae*). Journal of Phycology, 34(6): 918-924.
doi: 10.1046/j.1529-8817.1998.340918.x

9 - Perspectives

Table of contents

9.1 Further exploitations of the compiled time series of seabed mosaics

There are several clear interests in proceeding further with this imagery approach as a support of the study of distribution and dynamics of *Codium* populations and benthic communities, in Faial and elsewhere:

a. **Extending temporal monitoring: c**oncerning *Codium elisabethae*, on-going monitoring campaigns would allow to verify the hypothesis of cyclical regeneration process of density, or to confirm an actual general trend in population density reduction on the long term. Inter annual variations of its primary production could also be evaluated. In certain sites, it is known that populations are almost eliminated during exceptionally harsh winters (as in 1998 for the close by site of Baixa das Cabras, figure 4 in Chapter 2; Tempera, personal communication). Long term dynamic would deserve to be apprehended at the scale of the macroalga life span, as in complementary sites.

b. **New exploitations for additional key species dynamics** The image database compiled is a very rich source of information for the study of spatial and temporal distribution, density and biomass of other distinguishable species. As a matter of fact, during work focusing on *Codium elisabethae*, several other macroalga species (*Zonaria tournefortii, Asparagopsis armata*, the crustose *Coralinaceae*, *Padina pavonica*) but also the *Ascidiaceae Distalpia corolla*, sea urchins and sea stars) could already be clearly distinguished from the substrate on good images and spectral and texture information seems sufficient for image segmentation. In addition to these "high altitude" images used for the previous study (4-6 meters above seabed, including 4 reference plates for image registration), several complete image covers of the sites were also carried out as 'close up' (2 meters above seabed), allowing even smaller details and color differences to be distinguished (Fig. 1). The exploitation of these higher resolution images can produce far more information on the spatio-temporal dynamics of various species and of the communities, as well as a deeper insight on the dynamics of *Codium elisabethae* recruits, representing significant part of the primary productivity of this alga. The registration and geometric correction of these closer scale mosaics are possible by referencing them on the higher altitude mosaics already produced by simple identification of several corresponding points, or by pattern recognition. Such process would highly benefit of the input of a high resolution acoustic map of both Caldeirinhas and Ponta Furada reference sites.

c. **Improvement and integration of methodologies towards a friendly graphical interface.** It would be very interesting to continue improving and mainly integrating further the global image processing methodology allowing the establishment of precise spatially referenced time series of large sea bed visible mosaics, and their subsequent pre-processing according to various possible objectives expected by end-users. This tool could be used for the analysis of complementary mosaics available in the site of Caldeirinhas (similar analysis as in the present work to improve the statistic results, but also for the close cover analysis described above)

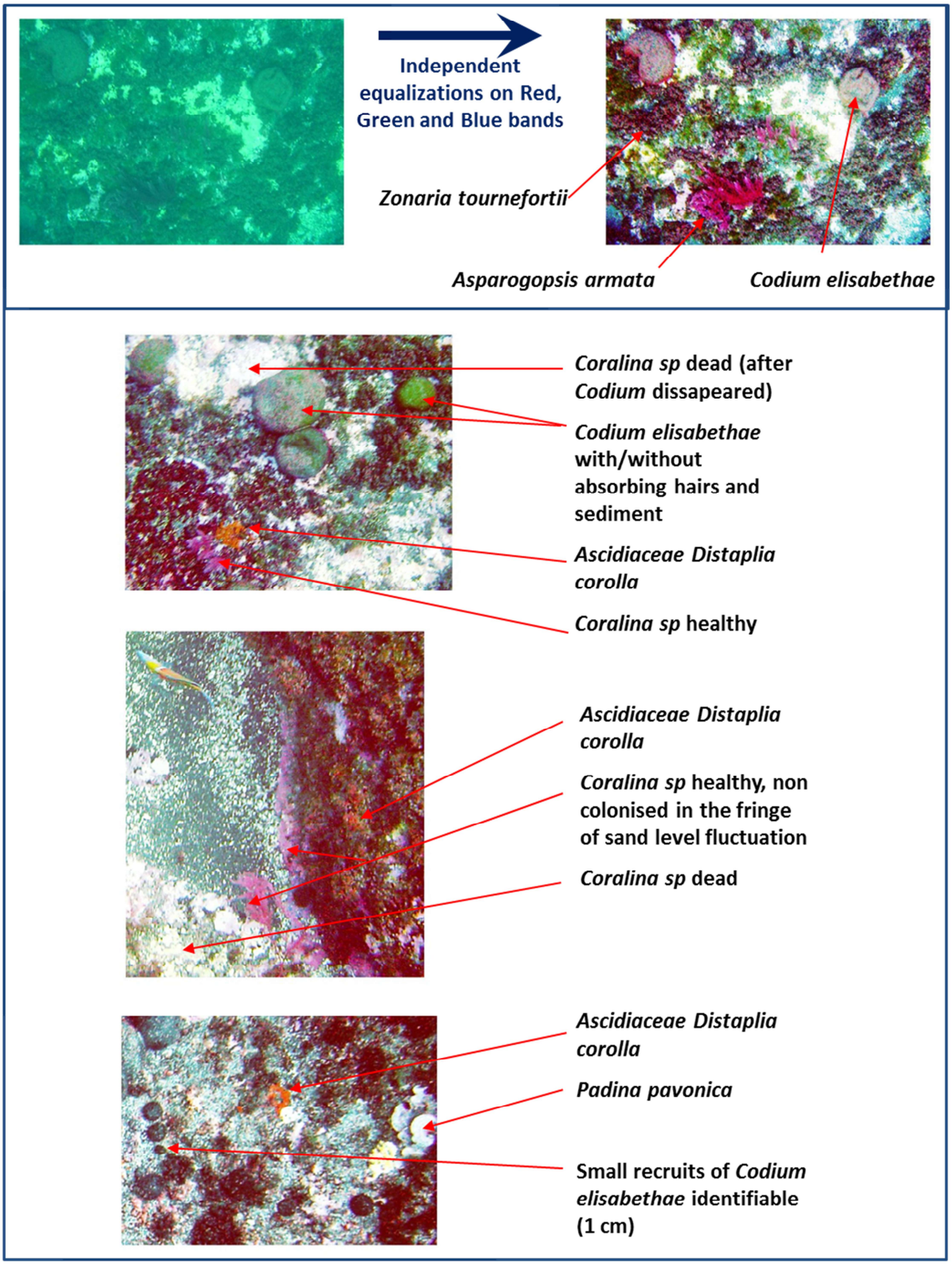


Figure 1 - Illustration of high spectral and spatial resolution information available in the 2 meters distant image coverage recorded; and potential exploitability for complementary studies on *Codium elisabethae*, other species, or macroalgae phytosociology.

9.2 Improvements of the imagery methodology

Numerous improvements can be foreseen:

1) Producing higher quality of mosaics registration based on faster, easier methods. They reside mainly in the acquisition of topographical information concerning long term stable reference marks, if possible in a more accurate way, but mainly in a faster approach than *in situ* distance measurements with metric lines subject to much difficulty of manipulation underwater due to currents, topography and the biota cover itself. Such improvements can be foreseen by the exploitation of rigid structures repeated in space for the definition of the grid of reference marks, by the use of high resolution acoustic mapping or positioning, or by advanced exploitation of video mosaicking solutions for 3D topography characterization.

2) Change detection could be improved by considering history presence on more than two successive mosaics to point towards possible relocation or problematic detection.

3) The increasing quality of still cameras (low light sensitivity, resolution) might allow new attempts to produce automatic detection based on combined exploitation of shape (texture information, as tested on early video imagery in Sirjacobs, 2002) and spectral information.

9.3 Priority questions concerning the population dynamic of *Codium elisabethae*

The final fate of propagules is still unstudied. Some might stay attached to parent plant as a substrate till decomposition of this last one, or might mostly finish by being detached by hydrodynamic actions and then fixating on the substrate and growing, or being advected away, dying and decomposing. The proportions of these possible pathways remain to be quantified to allow precise population dynamic modelling and understanding.

Their origin should also be investigated. From laboratory observation, propagules are shown to originate from two different processes: modification of the local shape of the spherical thallus which creates a small spherical extrusion or, more frequently, growth of a perfectly shaped small sphere looking like a young individual, with coenocyte filament entangled between the utricles of parent plant. Is this later case really a vegetative reproduction deriving from a filament which 'specializes', or is it simply a zygote that grew between utricles?

What is the survival time of gametes and germlings? To which distance can they be expected to be advected while still being able to contribute to reproduction and installation? How to quantify the degree of connectivity of populations between the two studied sites, between islands, between sub-regions. Entire studies should be devoted to understand the complexity of such benthic population's recruitment processes, as in Pineda *et al.* (2009). Future efforts should be devoted to integrate all the new information available at various scales (as evoked in section 3.4.3 and 3.4.4) to analyze global *C. elisabethae* population changes (and Mediterranean *Codium bursa* in parallel), with the new tools and databases existing (population dynamic from imagery, regional habitat population distribution from spatialized modelling, large scale biogeographical modelling with new climatologies?

9.4 Potential added value of underwater imagery for improving the maps of seaweeds habitat and rocky communities in Calvi Bay

In order to map density variations along the coastline and follow temporal evolution of macroalgal communities in the bay of Calvi (Corsica, France), Demoulin *et al.* (1980) and Janssens *et al.* (1993) integrated, at discrete survey stations, the qualitative visual estimation of algal coverage from surface, deep, and ascending/descending transects into average, minima and maxima values. In complement to this approach, the small scale spatial variations within each transects could be resolved by underwater imagery transects, allowing also some added value for long term quantitative change detection analysis based on conservation of visual records.

In another work in the Calvi Bay, Janssens (1993 and 2000) detailed the mean vertical zonation of macroalgae associations encountered in a short portion of coast ranging from Stareso harbor till the Cormoran's Rock. Resolving the horizontal spatial organization was not targeted in this approach, which aimed more at characterizing the mean depth distribution traits of the populations, making the hypothesis that they can be considered as a uniform unit concerning environmental context (solar exposition, exposition to hydrodynamics and to anthropic impacts). A reanalysis of that distribution with underwater imagery transects could reveal more local scale variability of the mean vertical zonation considered.

The production of a detailed vertical zonation with depth measurement of population limits over a 3D topographical representation would be overwhelmingly time consuming in scuba diving only. This both in reason of the complexity of manual topographical measurements underwater and because of the patchiness structure of algal vegetation. Nevertheless, a continuous 2D spatial representation of such vertical zonation (0-40m) could be produced along the same coast section with the help of well geo-located underwater digital imagery transects. This two dimensional information could be then projected over a shallow but high resolution seabed topography derived from acoustic mapping. Such information would provide a very useful basis for further interpretations and understanding of macroalgae spatial organization and dynamics in the shores of Calvi bay, as for the precise evaluation of their global biomass.

9.5 References

Demoulin, V., Janssen, M.P. and Licot, M., 1980. Mise au point d'une méthode de cartographie des macroalges marines: application à la région de Calvi (Corse). Lejeunia, 102: 1-68.

Janssens, M., 1993. Etat des peuplements algologiques de la baie de la Revellata (Calvi-Corse) : évolution, cartographie et biomasse. Mémoire de Maîtrise en Océanologie, Université de Liège, 71 pp.

Janssens, M., 2000. Etude *in situ* de la production primaire des macroalgues d'une baie méditerranéenne et influences dans le cycle du carbone : baie de la Revellata, Calvi (Corse - France). Thèse de Doctorat en Sciences, orientation botanique, Université de Liège, 270 pp.

Janssens, M., Hoffmann, L. and Demoulin, V., 1993. Cartographie des macroalgues dans la région de Calvi (Corse) : comparaison après 12 ans (1978-79, 1990-91). Lejeunia, 141: 62 pp.

Pineda, J., Reyns, N.B. and Starczak, V.R., 2009. Complexity and simplification in understanding recruitment in benthic populations. Population Ecology, 51: 17-32.
doi: 10.1007/s10144-008-0118-0

Sirjacobs, D., 2002. Matlab designed Image analysis software for detection of *Codium elisabethae* and calculation of population statistics. Summer training work realised within the MAROV Project *(*Mapping of Marine Habitats of the Azores using Robotic Ocean Vehicles*)*, co-ordinated by the Department of Oceanography and Fisheries, University of the Azores, Faial Island. ERASMUS grant report. Université de Liège, 31 pp. + Annexes.
http://www.horta.uac.pt/projectos/marov/reports/Final/Damien_2002.pdf

10 - Annexes

Table of contents

10.1 Sensors deployment periods

Caldeirinhas		
Sensors	**Deployment period**	
Minilogs T°C	installation	16/07/2004
	retrieval	13/08/2004
RCM9	installation	19/08/2004
	retrieval	23/11/2004
RCM9	installation	5/12/2004
	retrieval	3/03/2005
RCM9	installation	12/03/2005
	retrieval	24/06/2005
aquadopp	installation	18/07/2005
	retrieval	22/09/2005

Ponta Furada		
Sensors	**Deployment period**	
Minilogs T°C	installation	16/07/2004
	retrieval	13/08/2004
aquadopp	installation	19/08/2004
	retrieval	16/10/2004
aquadopp	installation	6/12/2004
	retrieval	3/03/2005
aquadopp	installation	16/03/2005
	retrieval	24/06/2005
RCM9	installation	21/07/2005
	retrieval	29/08/2005

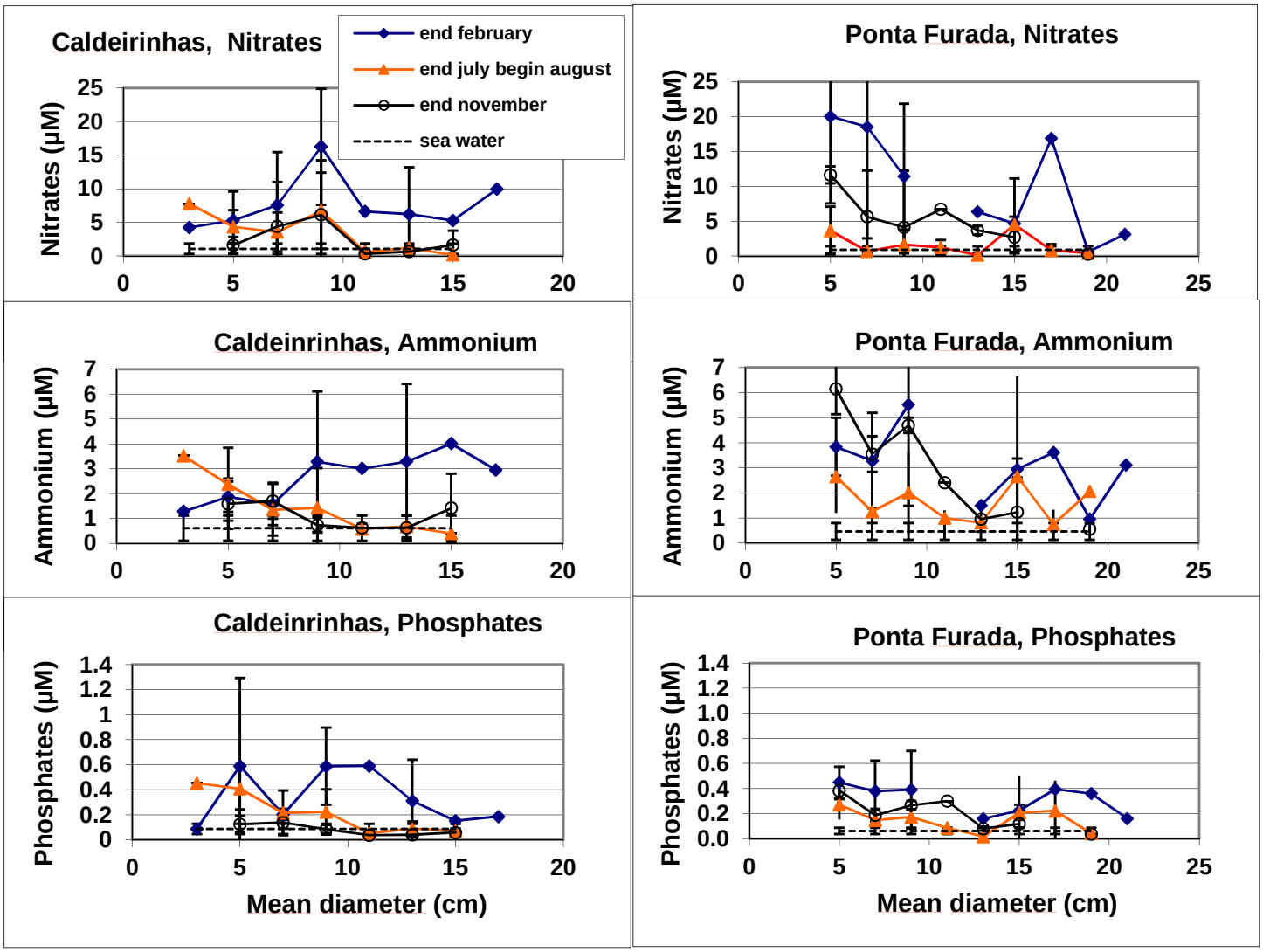
10.2 Variability of the density of utricles carrying gametangia on *Codium elisabethae*

The variability of the density of utricles carrying gametangia is presented in the table below, according to the upper, lateral, or down part sampled on the thallus of *Codium elisabethae.*

	Mean Horiz. Diam.	Density of utricules with gametangia		
Sample	**cm**	**DOWN**	**UP**	**LAT.**
Cgm19	15.4	26.0	20.0	38.0
Cgm 56	10.5	41.0	22.5	48.0
Cgm 57	10.3	0.0	25.0	26.0
Cgm 58	9.5	40.0	0.0	43.5
Cgm 66	6.5	2.0	1.0	18.0
Cgm 68	15.5	34.0	49.0	47.0
Cgm 69	20.0	47.0	58.5	44.0
Cgm 70	14.0	39.0	46.5	31.0
Cgm 71	14.5	0.0	43.0	0.0
Cgm 72	18.5	0.0	39.0	30.0
Cgm 73	15.0	46.0	0.0	38.0
Cgm 74	9.5	20.5	26.0	46.0
Cgm 75	13.3	0.0	64.0	30.0
	Mean G. density (%)	22.7	30.3	33.8
	Standard deviation of G. density (%)	19.7	21.8	13.7

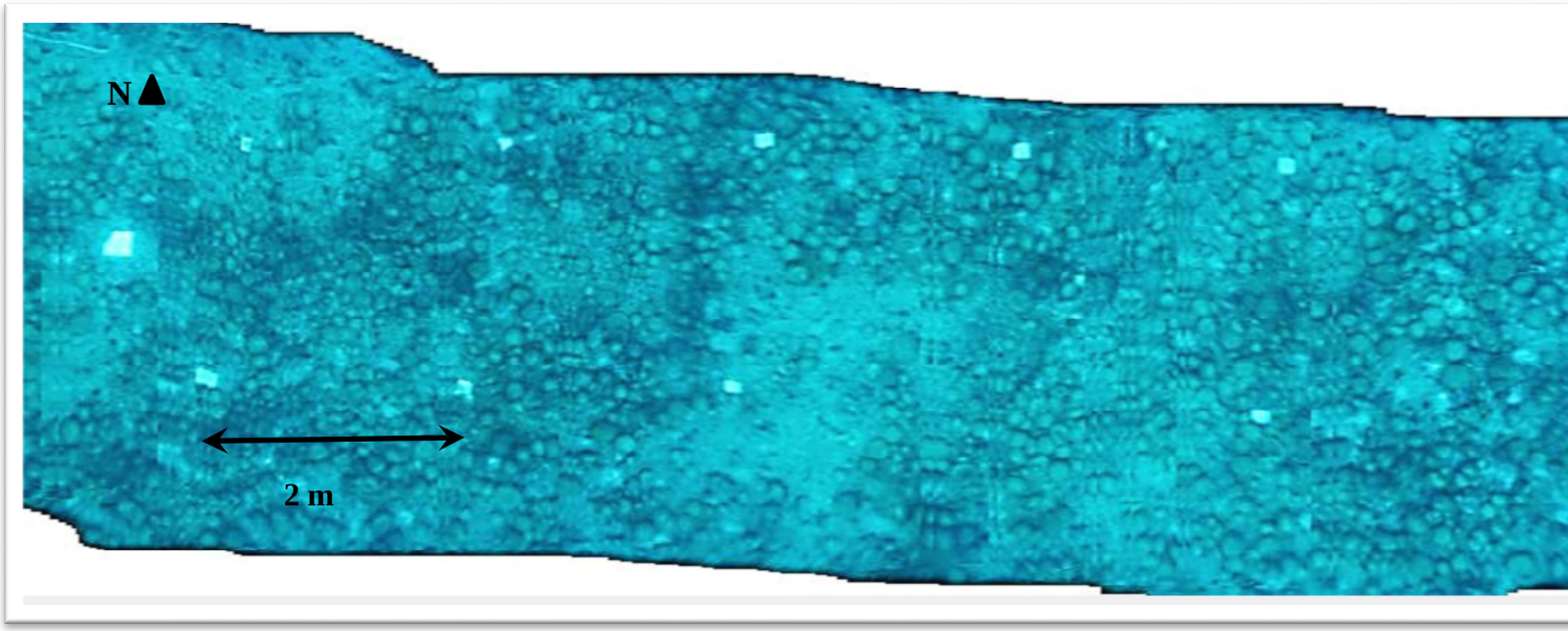
10.3 Details of nutrient concentrations in the lumen water

The figures below shows the lumen nutrient concentrations plotted per class size, per site and per season (Caldeirinhas in the left and Ponta Furada in the right)



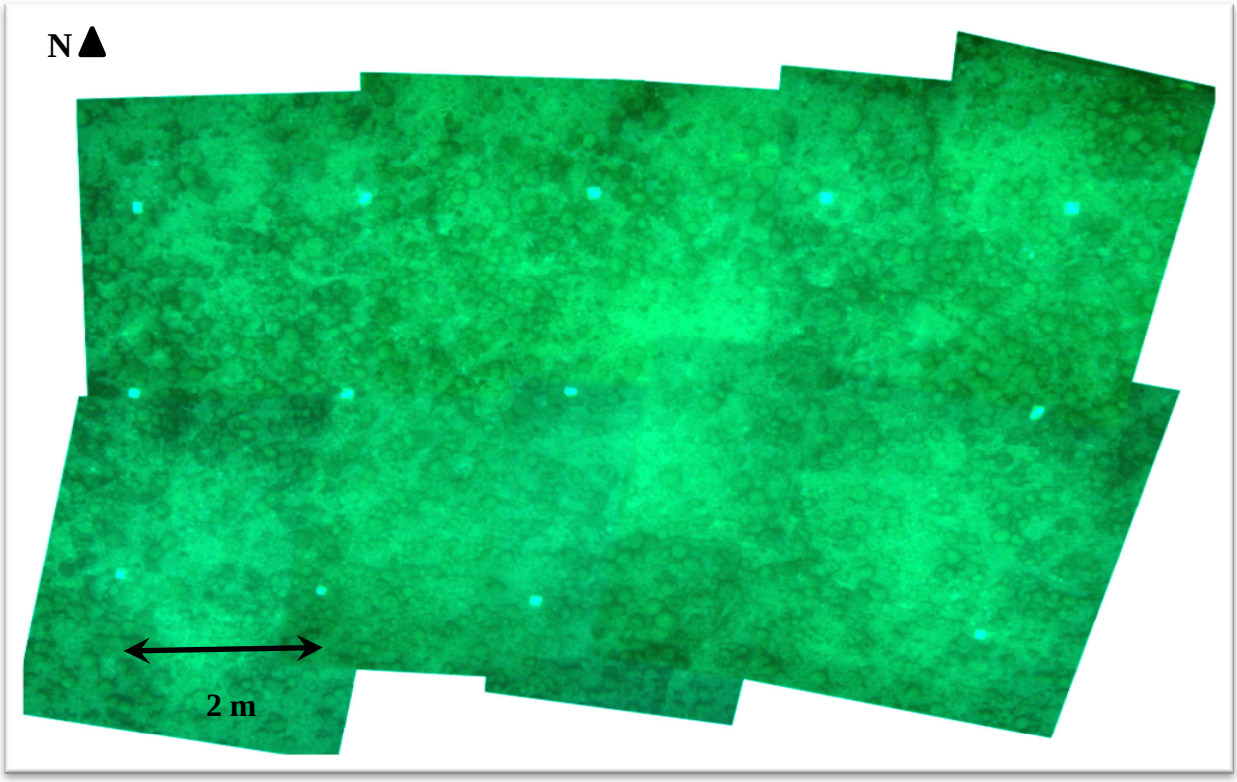
10.4 Videomosaic - Caldeirinhas

Example of video mosaic obtained with mosaico.m matlab code: easier process but occasional discontinuities result in problematic or impossible exploitation of the result in some areas, further, no scale and geometry are imposed and distortions can be important.

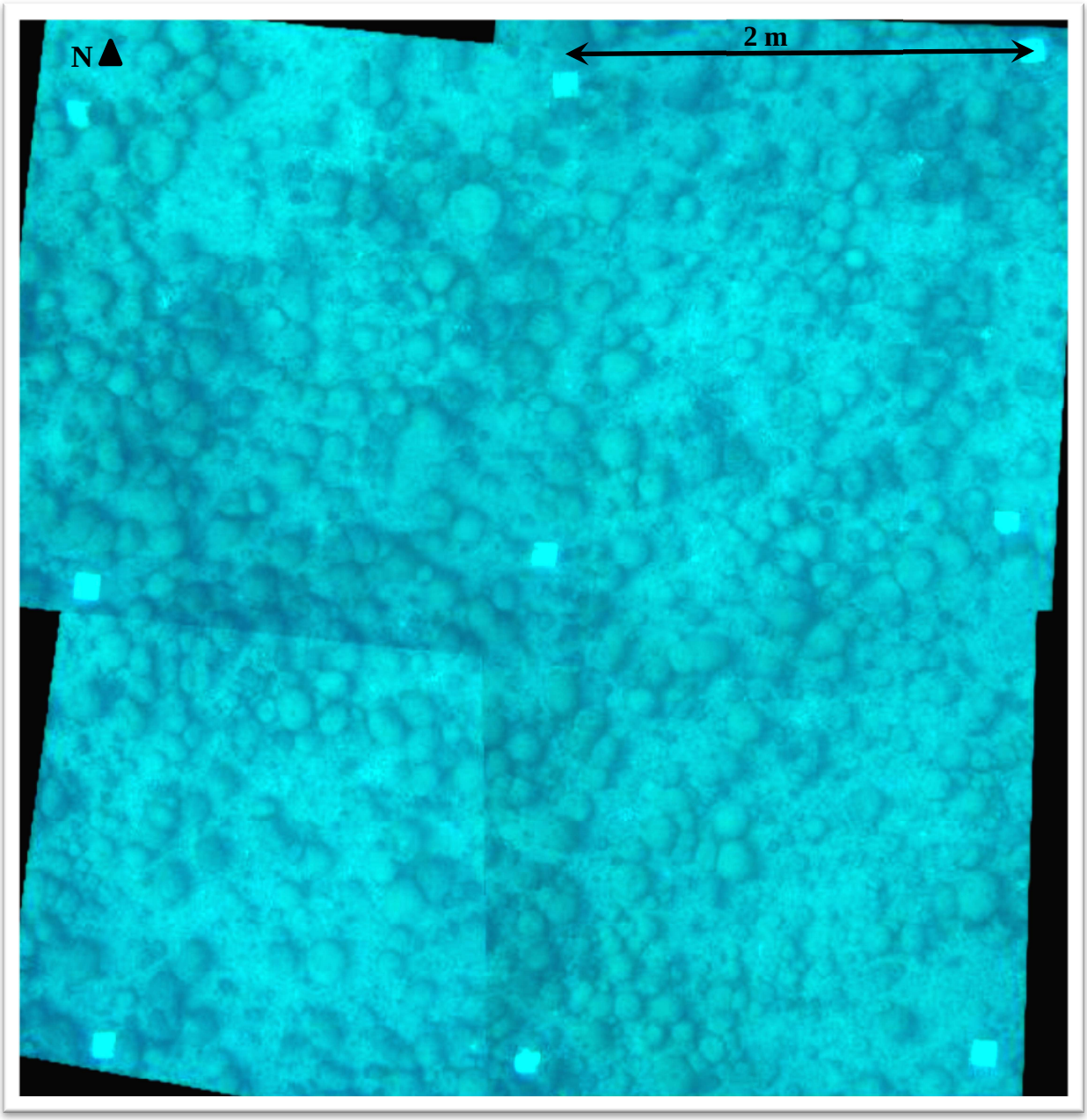


10.5 Photomosaic - Caldeirinhas (complete site)

Example of photo mosaic obtained with commercial stitching software: good image fusion and no discontinuities at edges still no scale and geometry imposed.

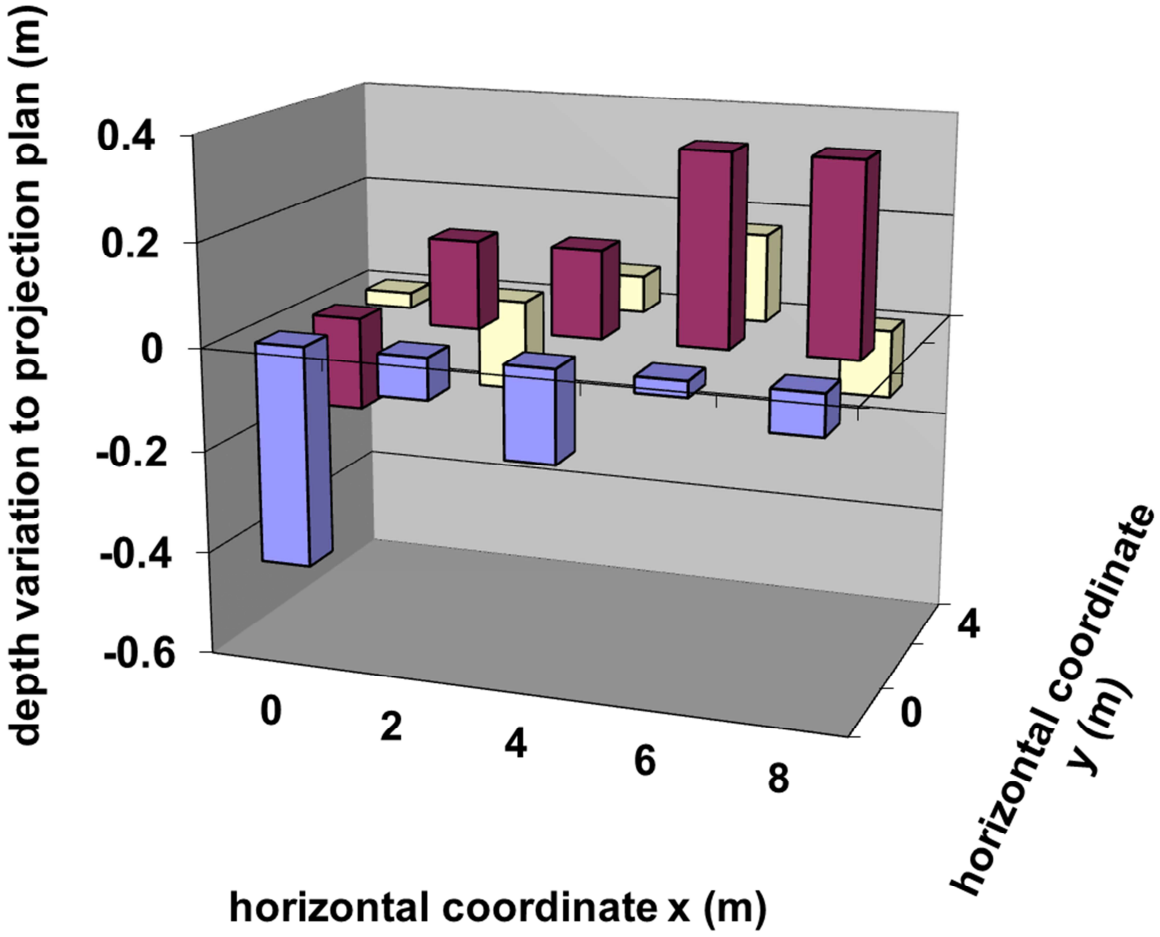


10.6 Photomosaic - Caldeirinhas (half site - 09/10/03)



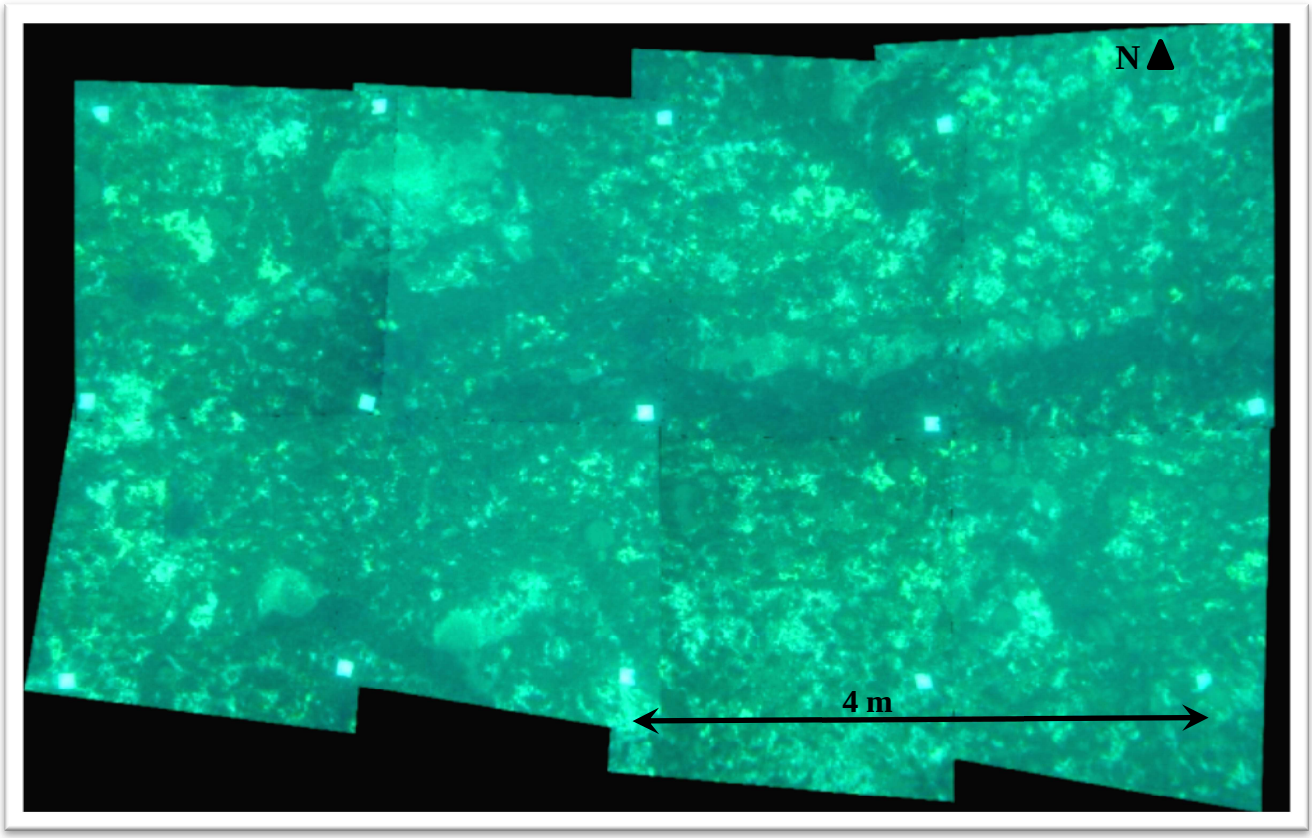
Example of photo mosaic obtained with matlab designed code: good image fusion at edges and position of the center of each reference mark imposed at known coordinates resulting from *in situ* measurements.

10.7 Topography considered in Ponta Furada

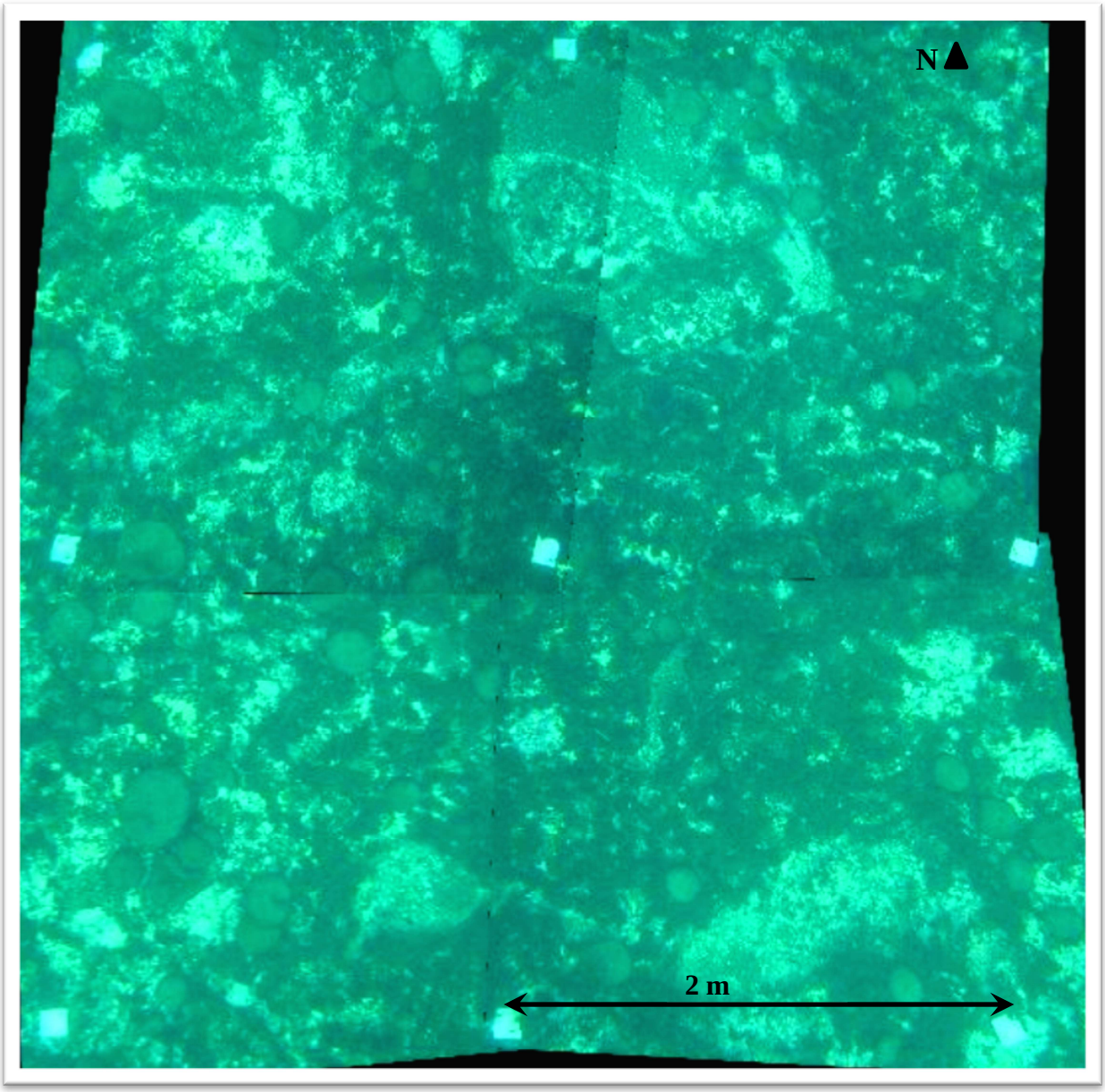


Depth variation around mean depth, network of reference marks of Ponta Furada, 27/07/05.

10.8 Complete photomosaic of the site of Ponta Furada



10.9 Photomosaic - Ponta Furada (half site - 26/11/04)



10.10 Contingency tables: imagery versus *in situ* counting methodologies

The contingency table used for testing the independence of the size histograms obtained by both methods (imagery versus *in situ*) is given below, aggregating all validation data for each site and globally, followed by the details of the statistical analysis.

	A		B		C	
	Cald.		**Ponta Furada**		**Cald and PF**	
max. diam.	**imagery**	***in situ***	**imagery**	***in situ***	**imagery**	***in situ***
1	0	37	0	15	0	52
2	0	46	0	9	0	55
3	9	61	0	2	9	63
4	34	51	1	10	35	61
5	28	33	1	4	29	37
6	25	29	5	6	30	35
7	19	20	5	9	24	29
8	11	14	5	7	16	21
9	13	11	4	7	17	18
10	11	10	2	2	13	12
11	13	13	3	4	16	17
12	11	13	10	9	21	22
13	4	7	4	5	8	12
14	8	8	12	11	20	19
15	4	5	8	7	12	12
16	3	2	4	6	7	8
17	0	0	3	4	3	4
18	1	1	4	3	5	4
19	0	0	3	1	3	1
20	0	0	5	8	5	8
21	0	1	6	4	6	5
22	2	0	2	3	4	3
23	0	1	2	2	2	3
24	1	1	3	2	4	3
25	0	0	0	1	0	1
26	0	0	0	0	0	0
27	0	0	1	1	1	1
28	0	0	0	0	0	0
29	0	0	0	0	0	0
30	0	0	0	0	0	0
31	0	0	1	1	1	1
32	0	0	2	3	2	3
33	0	0	2	1	2	1
34	0	0	0	0	0	0
35	0	0	0	0	0	0
36	0	0	0	0	0	0

Site of Caldeirinhas alone:

Testing equivalence for all size classes between 4 cm and 25 cm in diameter, no class aggregation or other data discard:
AEF (average expected frequency) = 317/(2*21)=7.54 > 6
Chi^2= 12.43; dl=20; p<0.99

Site of Ponta Furada alone:

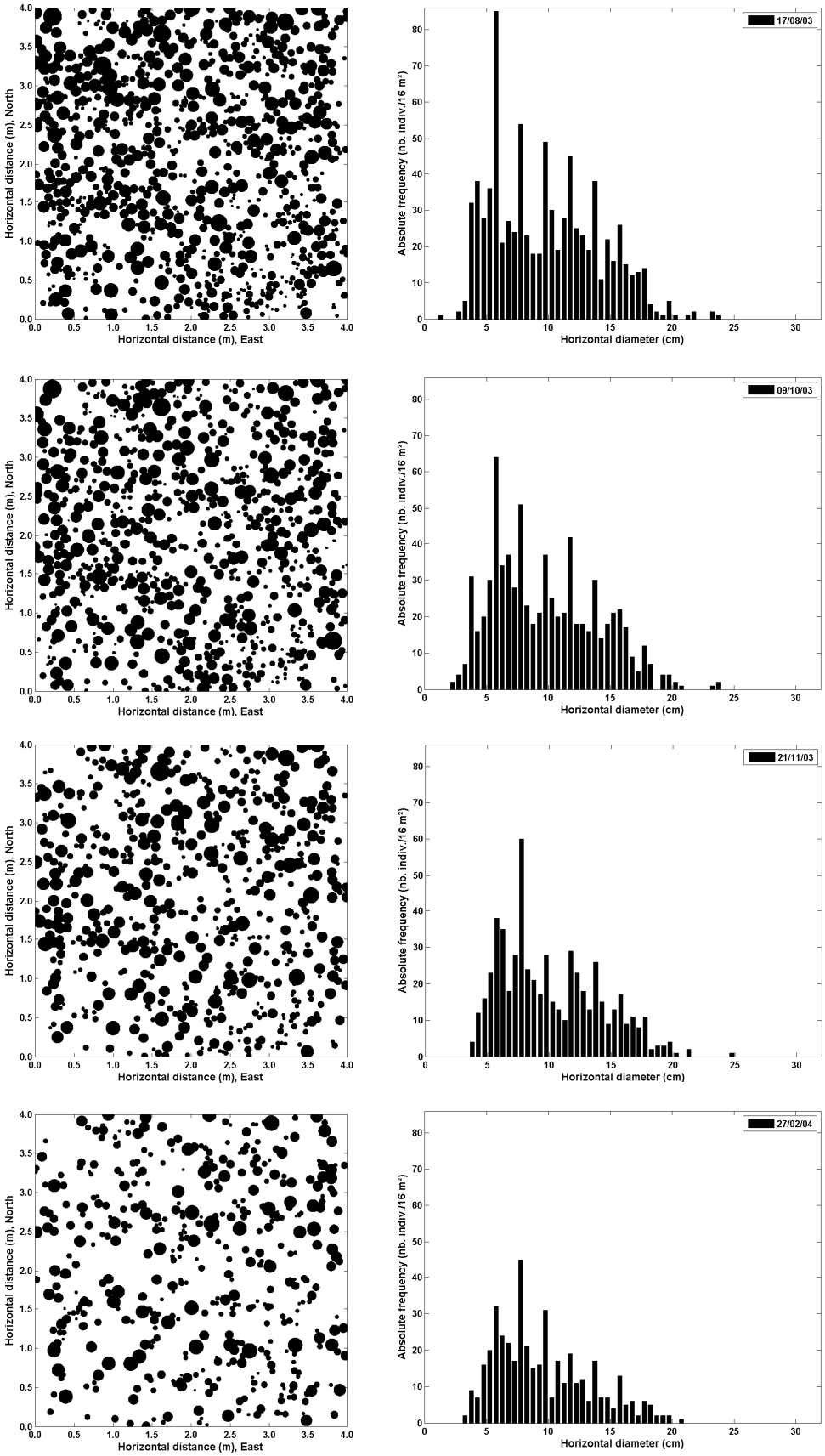
Testing equivalence for all size classes between 5 cm and 16 cm in diameter, no class aggregation, data above 16 cm discarded as too scarce and introducing bias (AEF condition rejected):
AEF for data above 5 cm = 203/(2*41)=2.47 < 6
AEF for data between 5 and 16 cm= 317/(2*21)=6.136 > 6
Chi^2= 5.263; dl=10; p<0.873

Data from both sites aggregated:

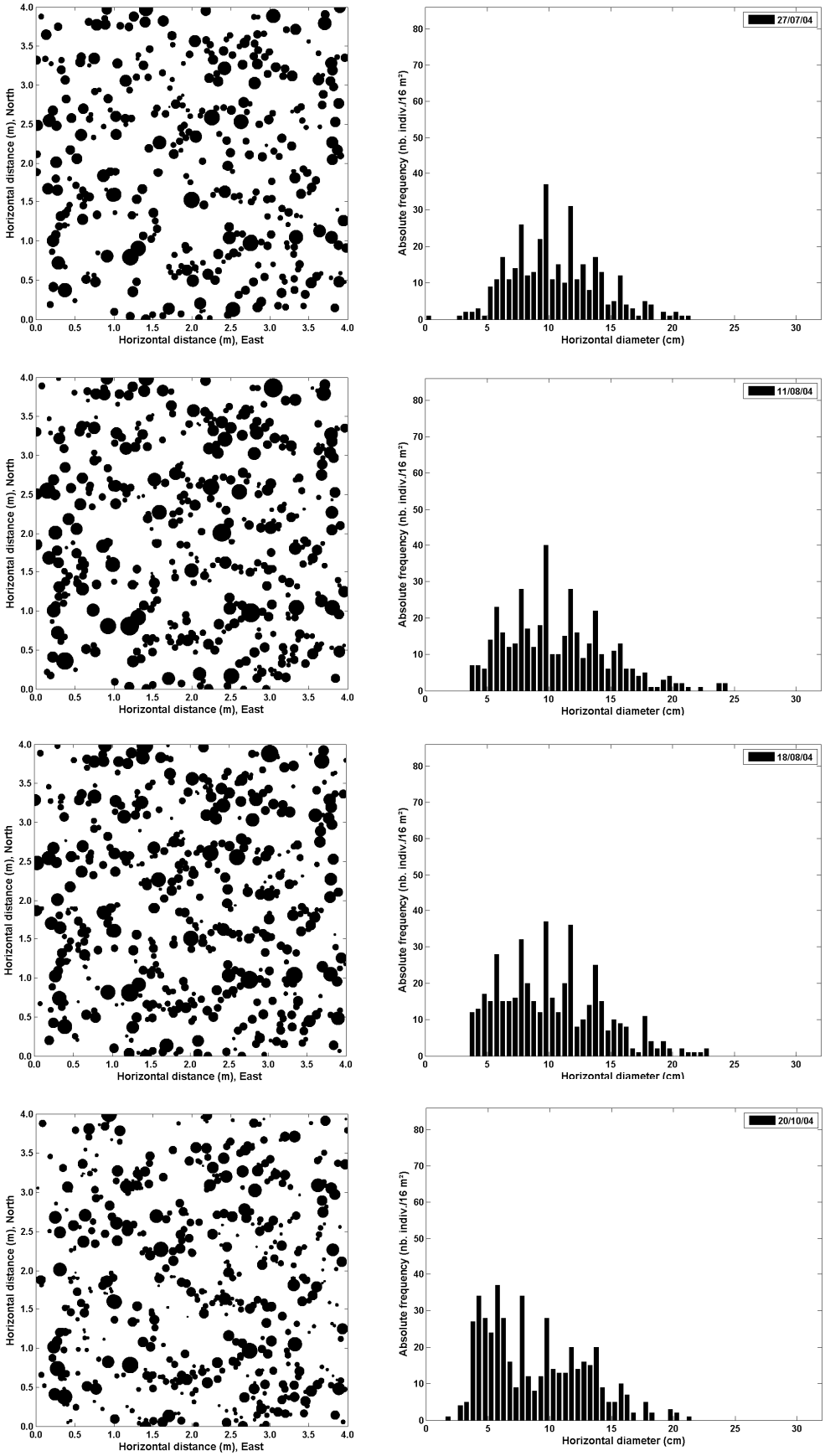
Testing equivalence for all size classes above 4 cm, no class aggregation or other data discard
AEF for data between 4 and 34 cm= 531/(2*30)=8.85 > 6
Chi^2= 15.409; dl=29; p<0.981

10.11 Detection results in Caldeirinhas population

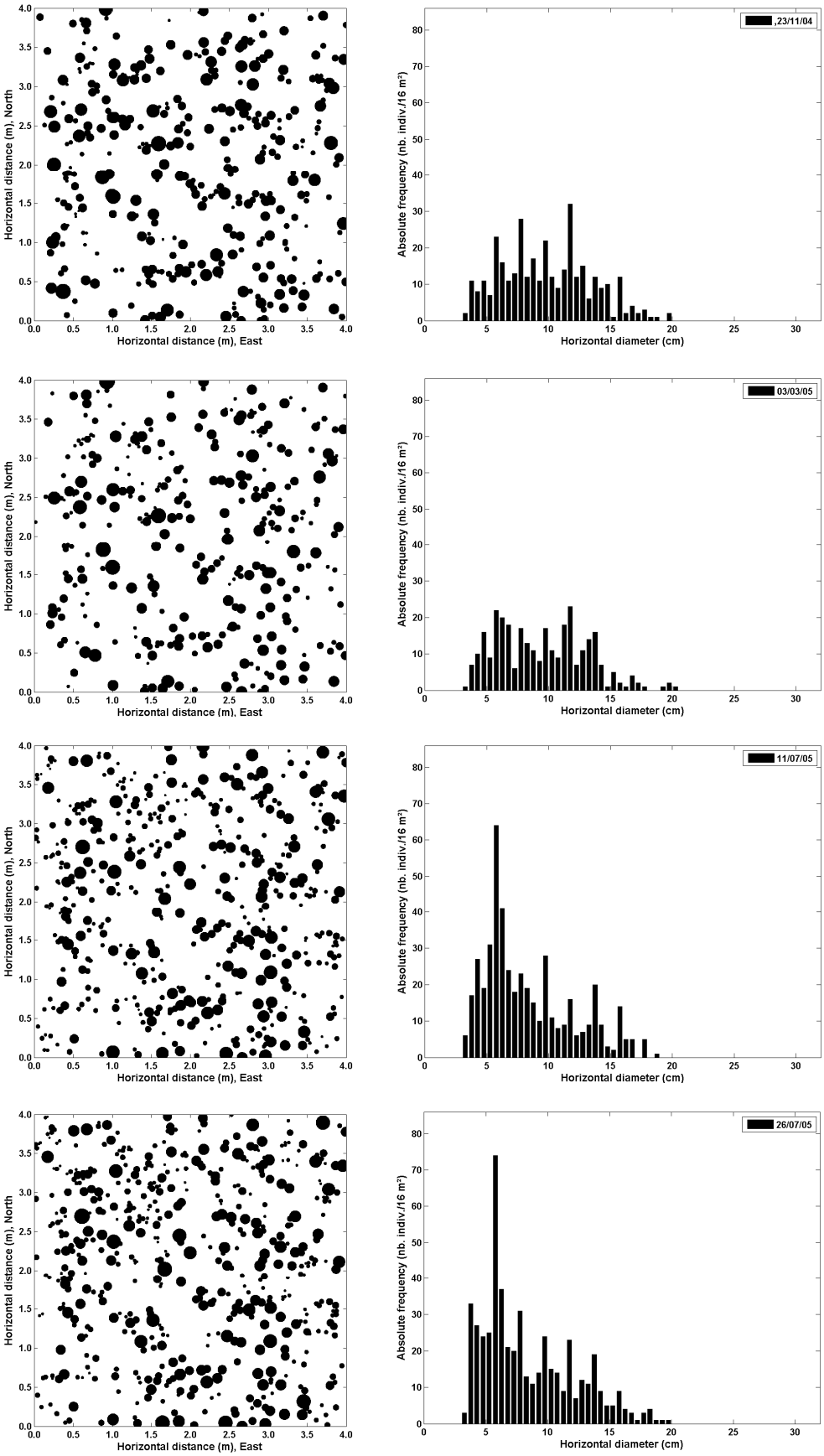
The detection results of *Codium elisabethae* population distribution and size histograms are illustrated for 16 m² in chronological order in the next 4 pages, for all seabed images mosaics exploited over the half of the site, representing 4 by 4 m zone. Individuals are identified in black, on the left column (1pixel = 2 mm). Histograms are on the right column with class size i/n horizontal axis (cm) and total number of individuals in vertical axis.



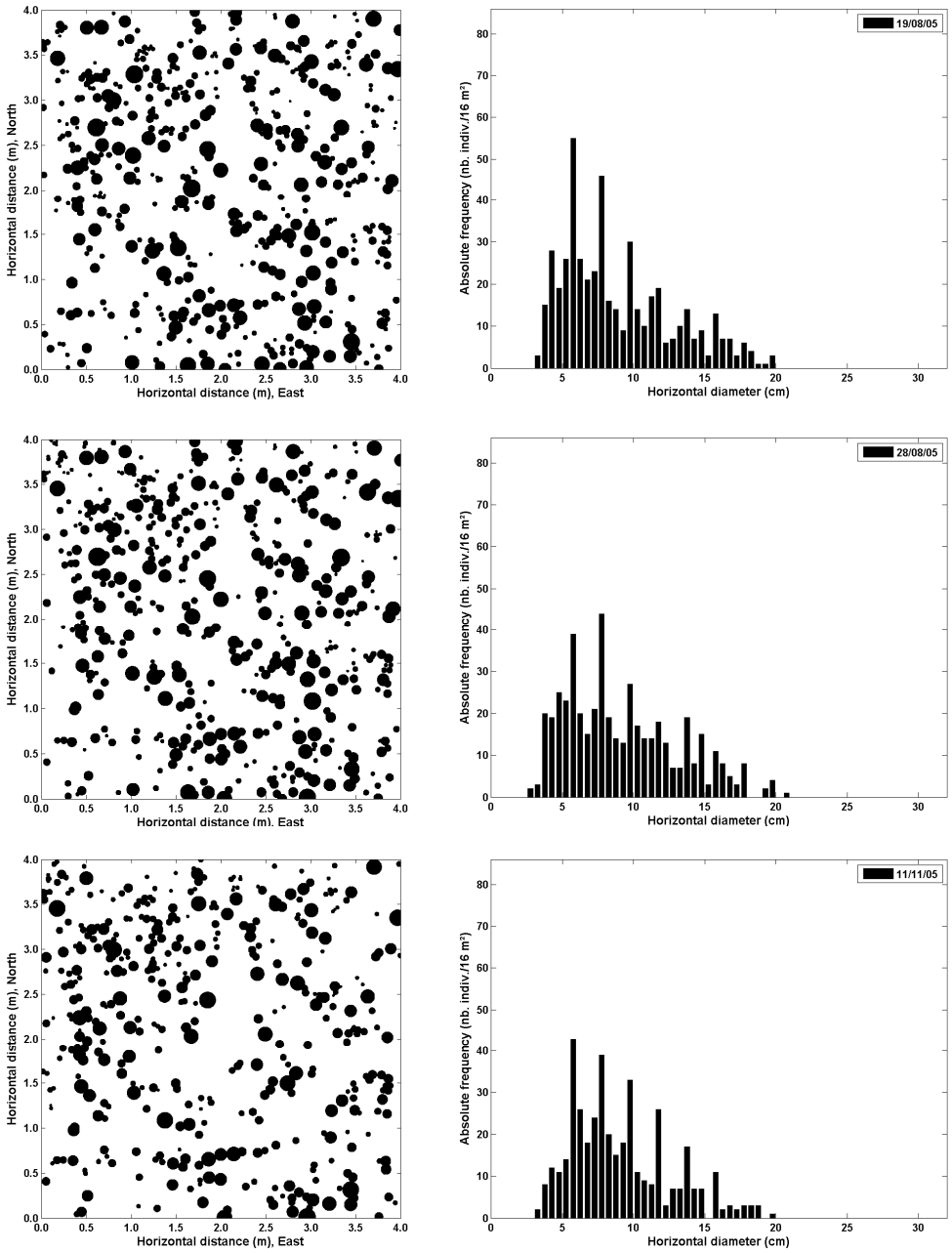
Time series (top to down: 17/08/03; 09/10/03; 21/11/03; 27/02/04) of the distribution (left) and size histogram (right) of *Codium elisabethae* population within a 16 m² mosaic of the Caldeirinhas site.



Time series (top to down: 27/07/04; 11/08/04; 18/11/04; 20/10/04) of the distribution (left) and size histogram (right) of *Codium elisabethae* population within a 16 m² mosaic of the Caldeirinhas site.



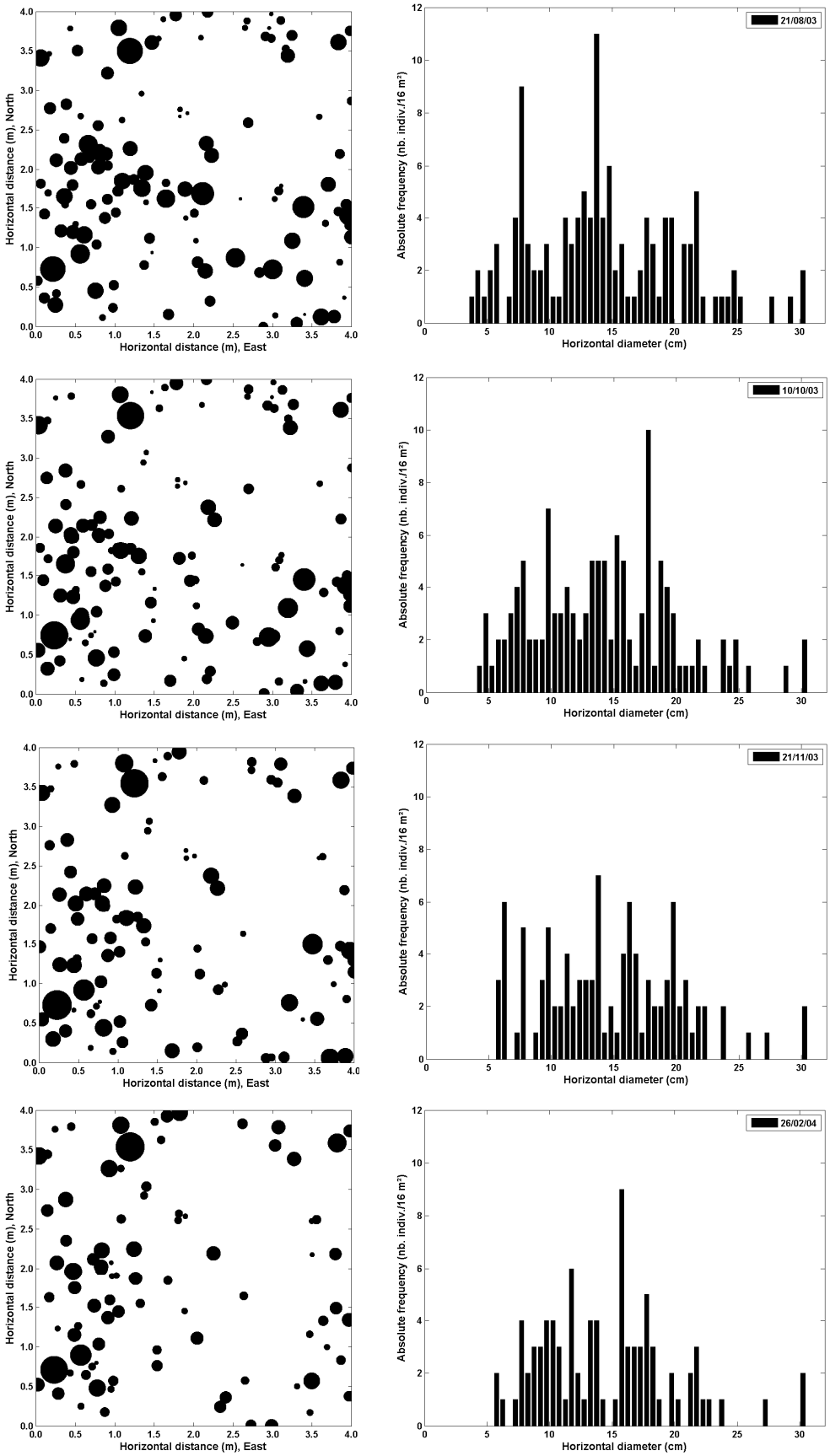
Time series (top to down: 23/11/04; 03/03/05; 11/07/05; 26/07/05) of the distribution (left) and size histogram (right) of *Codium elisabethae* population within a 16 m² mosaic of the Caldeirinhas site.



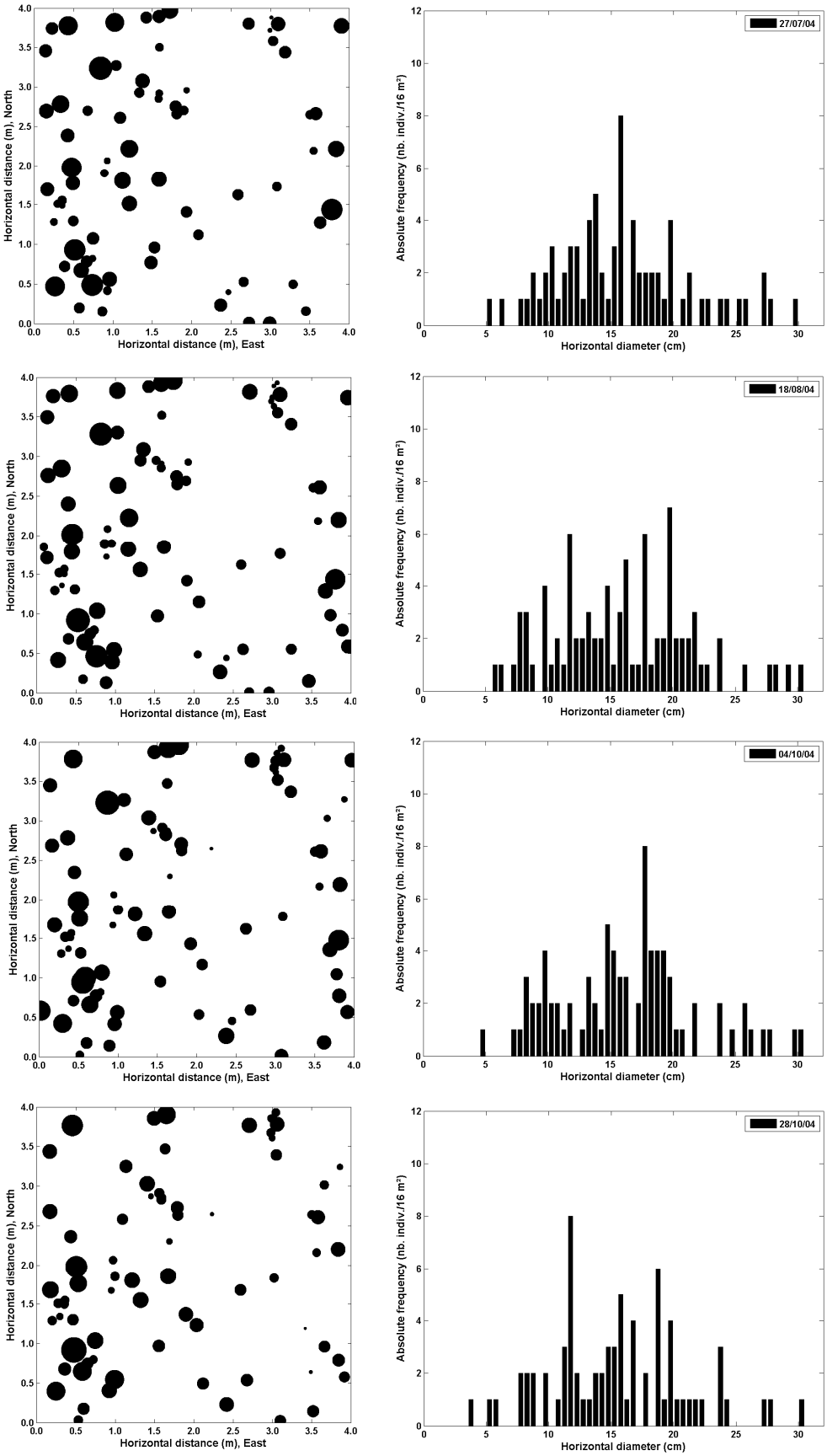
Time series (top to down: 19/08/05; 28/08/05; 11/11/05) of the distribution (left) and size histogram (right) of *Codium elisabethae* population within a 16 m² mosaic of the Caldeirinhas site.

10.12 Detection results in Ponta Furada population

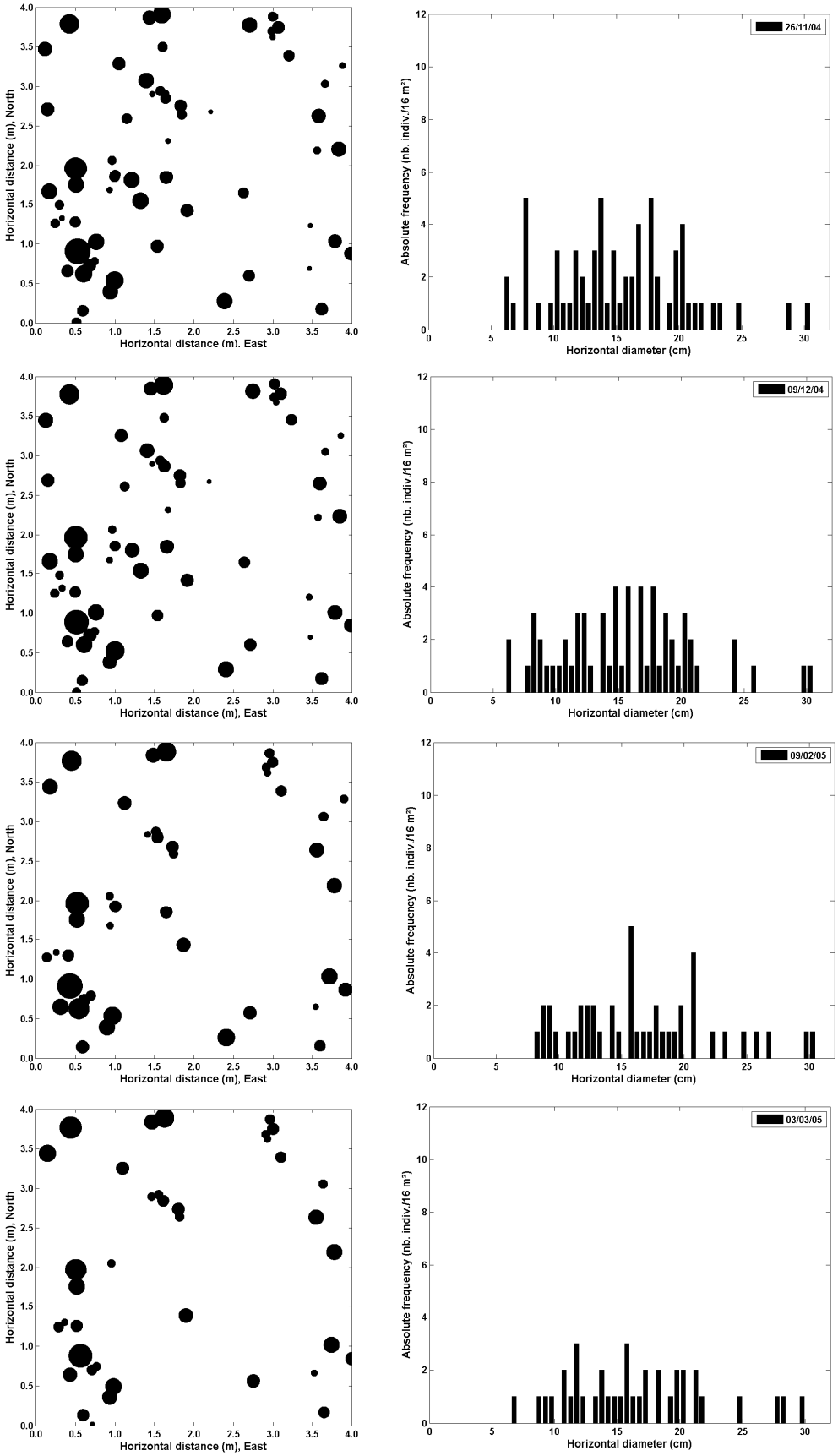
The detection results of *Codium elisabethae* population distribution and size histograms are presented in the next 5 pages in chronological order, for all seabed images mosaics exploited over the half of the site, representing 4 by 4 m zone. Individuals are identified in red, on the left colmun with pixels rows and lines as axis (1pixel= 5mm). Histograms are on the right column with class size in horizontal axis (cm) and total number of individuals in vertical axis.



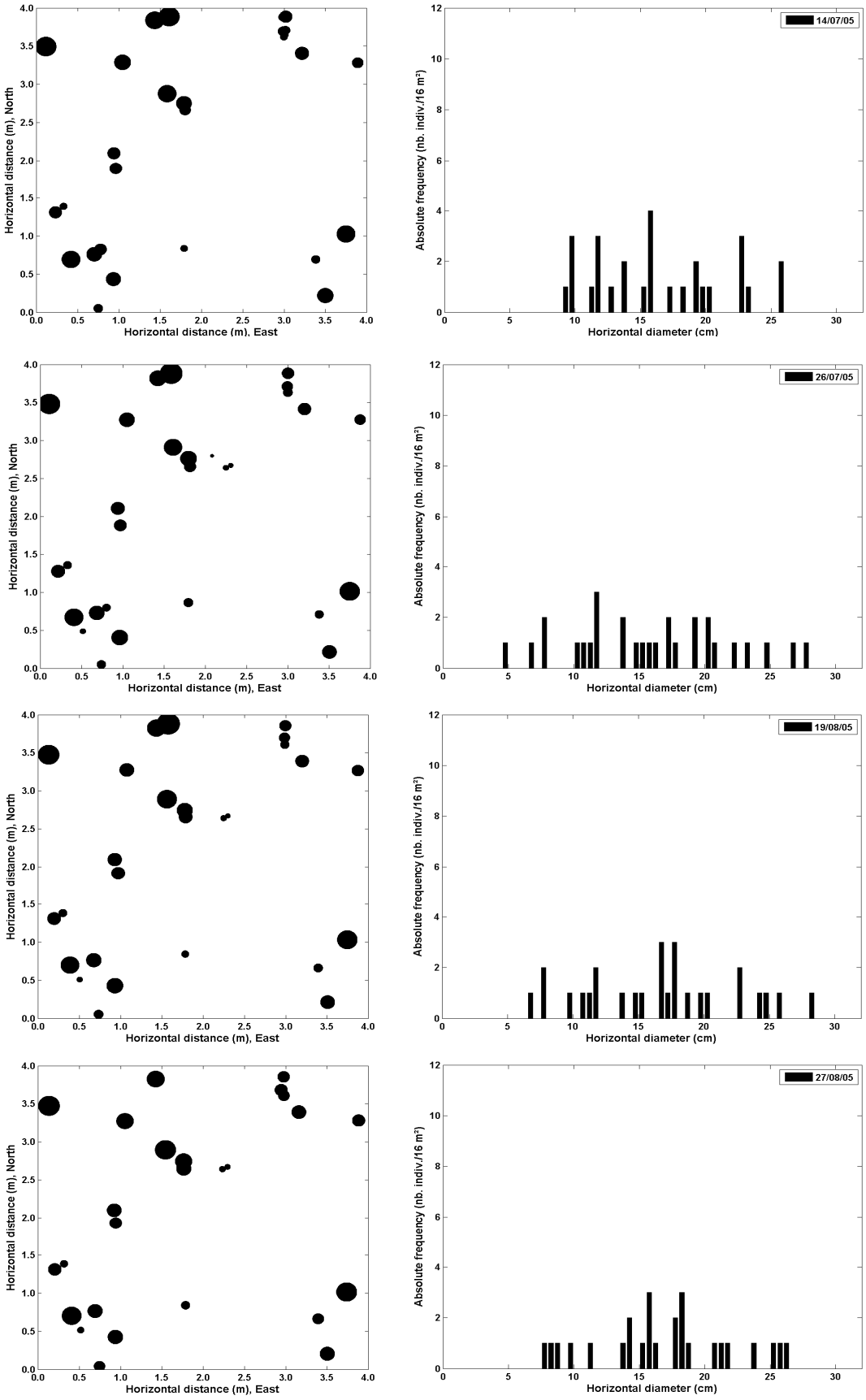
Time series (top to down: 21/08/03; 10/10/03; 21/11/03; 26/02/04) of the distribution (left) and size histogram (right) of *Codium elisabethae* population within a 16 m² mosaic of the Ponta Furada site.



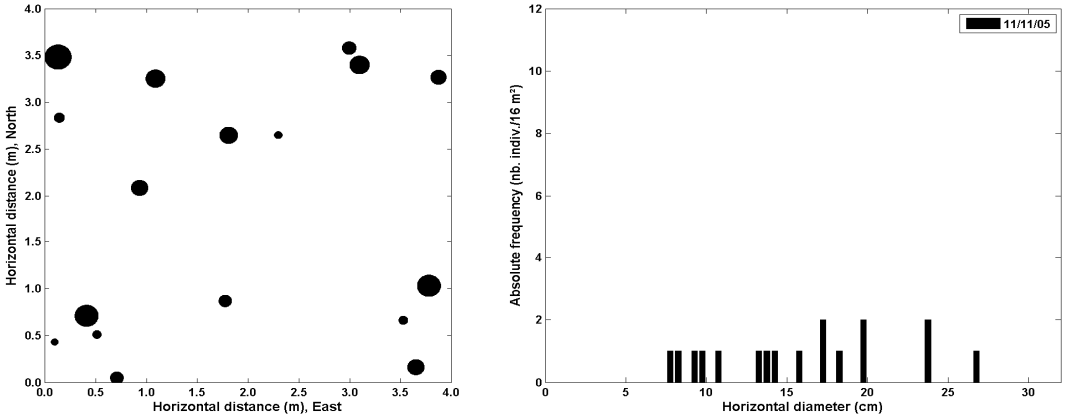
Time series (top to down: 27/07/04; 18/08/04; 04/10/04; 28/10/04) of the distribution (left) and size histogram (right) of *Codium elisabethae* population within a 16 m² mosaic of the Ponta Furada site.



Time series (top to down: 26/11/04; 09/12/04; 09/02/05; 03/03/05) of the distribution (left) and size histogram (right) of *Codium elisabethae* population within a 16 m² mosaic of the Ponta Furada site.



Time series (top to down: 14/07/05; 26/07/05; 19/08/05; 27/08/05) of the distribution (left) and size histogram (right) of *Codium elisabethae* population within a 16 m² mosaic of the Ponta Furada site.



Distribution (left) and size histogram (right) of *Codium elisabethae* population observed on the 11/11/05 within a 16 m² mosaic of the Ponta Furada site.